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(71) Applicant *(for all designated States except US)*: **ELI LILLY AND COMPANY** [US/US]; Lilly Corporate Center, Indianapolis, IN 46285 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **SU, Eric, Wen** [US/US]; 13447 Dunes Drive, Carmel, IN 46032 (US). **NA, Songqing** [CN/US]; 10756 Independence Way, Carmel, IN 46032 (US).

(74) Agents: **SHARP, Robert, L.** et al.; Eli Lilly and Company, Lilly Corporate Center, Indianapolis, IN 46285 (US).

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(54) Title: HUMAN FGF-20 NUCLEIC ACIDS AND POLYPEPTIDES

(57) Abstract: The present invention relates to human FGF-20 (hFGF-20) polypeptides, and isolated nucleic acids related thereto. Vectors, host cells, transgenics and chimeric mammals comprising hFGF-20 polynucleotides, and human FGF-20 recognizing antibodies, as well as methods of making and using them, are also included in the present invention. The invention is also directed to compositions for modulating, diagnostic methods for detecting, and therapeutic methods for inhibiting, the hyperproliferation of cells and formation, development and maintenance of neurological disorders or tumors.

HUMAN FGF-20 NUCLEIC ACIDS AND POLYPEPTIDES

BACKGROUND OF THE INVENTION

FIELD OF THE INVENTION

This invention relates to recombinant DNA technology. In particular the invention relates to compounds and pharmaceutical compositions comprising novel human fibroblast growth factor-20 (hFGF-20) polypeptides and nucleic acids. The present invention also includes methods of making and using, both diagnostically and therapeutically, hFGF-20 nucleic acids, polypeptides, hFGF-20 epitope recognizing antibodies as well as host cells, transgenics, and chimerics comprising hFGF-20 nucleic acids.

RELATED ART

Stroke is the third leading cause of death and disability in the United States. With an excess of 500,000 new occurrences each year, strokes are responsible for about 300,000 deaths annually in the U.S. alone. Strokes are also a leading cause of hospital admissions and long-term disabilities. Accordingly, the socioeconomic impact of stroke and its attendant burden on society is immense.

"Stroke" is defined by the World Health Organization as a rapidly developing clinical sign of focal or global disturbance of cerebral function with symptoms lasting at least 24 hours. More than 85% of strokes are due to a thrombo-embolic occlusion of a cerebral artery, resulting in death of brain tissue. With complete occlusion, arrest of cerebral circulation causes cessation of neuronal electrical activity within seconds. Within a few minutes after the deterioration of the energy state and ion homeostasis, depletion of high energy phosphates, membrane ion pump

failure, efflux of cellular potassium, influx of sodium chloride and water, and membrane depolarization occur. If the occlusion persists for more than five to ten minutes, irreversible damage results. Although severe ischemia can be lethal, generally, ischemia is not total after a thrombotic occlusion of a cerebral vessel. Even with incomplete ischemia, however, the outcome is difficult to evaluate and depends largely on residual perfusion and the availability of oxygen.

When the ischemic event is moderate it generally results in a region of collaterally perfused tissue having paralyzed neuronal function surrounding a core of severe ischemia where the infarct developed. The zone of damaged brain tissue, called a "penumbra", consists of brain tissue in a state between life and death. Oftentimes, the loss of neuronal function can be reversed with restoration of adequate perfusion.

Cerebral infarction commonly results in deficits of motor, sensory, visual, or cognitive function. Recovery involves dendritic and axonal sprouting and new synapse formation in the damaged tissue or other hemisphere. The eventual extent of neurologic recovery depends on the patient's age and general state of health as well as on the site and size of the infarction. Impaired consciousness, mental deterioration, or aphasia, suggest a poor prognosis. About 50% of patients with moderate or severe hemiplegia, and most of those with lesser deficits, recover functionally and are ultimately able to care for their basic needs, have a clear sensorium, and can walk adequately, although use of an affected limb may be limited. Although some patients continue to improve slowly, any deficit remaining after 6 months is likely to be permanent. Furthermore, recurrence of cerebral infarction is relatively common, and each recurrence is likely to add to the neurologic disability.

In addition to stroke, other neurological diseases are also associated with the death of or injury to neuronal cells. For example, Parkinson's disease results from the loss of dopaminergic neurons in the substantia nigra. Although the molecular mechanism of neurodegeneration in Alzheimer's disease is yet to be established, inflammation and deposition of beta-amyloid protein and other such agents may compromise neuronal function or survival. In patients suffering from brain trauma or spinal cord injuries, extensive neuronal cell death is also observed. Nitric oxide (NO) and oxidative stress are thought to play important roles in the pathogenesis of many neurodegenerative disorders including neuronal dysfunction after stroke or head trauma, retinal degeneration, Alzheimer's disease and Parkinson's disease. NO also plays a role in neuropathic pain, migraine, psychoses, angiogenesis, and vasculogenesis.

Although there are presently no satisfactory treatments for these diseases the use of compounds capable of inhibiting neuronal cell death, promoting nerve regeneration, stimulating neurite outgrowth, and inhibiting NO mediated toxicity are promising approaches to treatment of such disorders. In individuals suffering from a neurological disease, an induction of neurite outgrowth may protect neurons from further degeneration, and accelerate the regeneration of nerve cells. Neurite outgrowth may be stimulated in vitro by various growth factors. For example, Glial Cell Line-Derived Neurotrophic Factor (GDNF) demonstrates neurotrophic activity both, *in vivo* and *in vitro*, and is currently being investigated for the treatment of Parkinson's disease. Insulin and insulin-like growth factors have been shown to stimulate growth of neurites in rat pheochromocytoma PC12 cells and in cultured sympathetic and sensory neurons (Recio-Pinto et al., J. Neurosci.,

6:1211-1219 (1986)). Similarly, fibroblast growth factor-2 (bFGF) stimulates neural proliferation and growth (D. Gospodarowicz et al., Cell Differ., 19:1 (1986); M. A. Walter et al:, Lymphokine Cytokine Res., 12:135 (1993)), enhances neuronal sprouting *in vitro*, and stimulates functional recovery in neuronal recuperation models (Kawamata et al., J. Cereb. Blood Flow Metab., 16:542-547, (1996); Kawamata et al., Proc. Natl. Acad. Sci. USA, 94:8179:8184, (1997a)). Likewise, FGF-8 has been shown to play a critical role in growth and polarity of the developing midbrain and also has direct biological activities on neurons, including increasing GABA uptake and CREB phosphorylation in rat cortical cultures (Lee et al. 1997) Green et al. 1998). FGF-9 is also thought to play an important role in the normal and abnormal functioning of the central nervous system (Miyamoto, M., et al., Mol. and Cell. Bio., 13(7):4251-4259 (1993); Todo, T., et al., Brain Research, 783(2):179-87 (1998); Todo, T., et al., Neurosurgery, 43(2):337-46 (1998); Nakamura, S., et al., Glia, 28:53-65 (1999); Nakamura, S., et al., Brain Research 814:222-225 (1998)).

SUMMARY OF THE INVENTION

The present invention provides isolated hFGF-20 nucleic acids and hFGF-20 polypeptides encoded thereby, including fragments and specified variants thereof. Contemplated by the present invention are hFGF-20 probes, primers, vectors, host cells, transgenics, chimerics, hFGF-20 epitope recognizing antibodies, as well as methods of making and using them diagnostically and therapeutically, as described and enabled herein.

The present invention includes an isolated nucleic acid molecule comprising a polynucleotide that encodes an hFGF-20 polypeptide as defined herein, as well as specified variants

thereof, or a nucleic acid molecule that is complementary to any such polynucleotide.

The present invention further provides recombinant vectors, comprising 1-40 of said isolated hFGF-20 nucleic acid molecules of the present invention, host cells containing said nucleic acids and/or said recombinant vectors.

The present invention also provides methods of making or using hFGF-20 nucleic acids, as well as, vectors, host cells, and transgenic animals comprising said nucleic acids. Such uses of hFGF-20 nucleic acids include, but are not limited to, the production of hFGF-20 nucleic acids and/or polypeptides by recombinant, synthetic, and/or purification techniques known in the art.

A polypeptide of the present invention includes an isolated hFGF-20 polypeptide comprising at least one fragment, domain, or specified variant having over its entire length at least 90-100% of the corresponding contiguous amino acids shown in either SEQ ID NOS:4 or 5.

In another embodiment the present invention relates to an isolated polypeptide, or functional fragment thereof, wherein said polypeptide comprises the sequence identified as SEQ ID NO:4. Examples of functional fragments of preference include polypeptides comprising SEQ ID NO:4 wherein said polypeptide lacks from 1 to 50 amino acid residues from the amino terminus of SEQ ID NO:4 or from 1 to 260 amino acid residues from the carboxy-terminus of SEQ ID NO:4. More preferably, functional fragments are polypeptides comprising SEQ ID NO:4 wherein said polypeptide lacks from 10 to 25 amino acid residues from the amino-terminus of SEQ ID NO:4 and from 1 to 260 amino acid residues from the carboxy-terminus of SEQ ID NO:4. A most preferred functional fragment is the polypeptide as shown in SEQ ID NO:5.

The present invention also provides an isolated nucleic acid probe, primer, or fragment, as described herein, wherein the nucleic acid comprises a polynucleotide of at least 10 nucleotides, corresponding or complementary to at least 10 nucleotides of at least one of SEQ ID NOS:1, 2, or 3.

The present invention also provides a recombinant vector comprising an isolated hFGF-20 nucleic acid as described herein.

The present invention also provides a host cell, comprising an isolated hFGF-20 nucleic acid as described herein.

The present invention also provides a method for constructing a recombinant host cell that expresses an hFGF-20 polypeptide, comprising introducing into the host cell an hFGF-20 nucleic acid in replicatable form as described herein to provide the recombinant host cell. The present invention also provides a recombinant host cell provided by a method as described herein.

The present invention also provides a method for expressing at least one hFGF-20 polypeptide in a recombinant host cell comprising culturing a recombinant host cell as described herein under conditions wherein at least one hFGF-20 polypeptide is expressed in detectable or recoverable amounts.

The present invention also provides an isolated hFGF-20 polypeptide produced by a recombinant, synthetic, and/or any suitable purification method as described herein and/or as known in the art.

The present invention also provides an hFGF-20 antibody or fragment, comprising a polyclonal and/or monoclonal antibody or fragment that specifically binds at least one epitope specific to at least one hFGF-20 polypeptide as described herein.

The present invention also provides a method for producing an hFGF-20 antibody or antibody fragment, comprising generating the antibody or fragment that binds at least one epitope that is specific to an isolated hFGF-20 polypeptide as described herein, the generating done by known recombinant, synthetic and/or hybridoma methods.

The present invention also provides an hFGF-20 antibody or fragment produced by a method as described herein or as known in the art.

The present invention also provides a composition comprising an isolated hFGF-20 nucleic acid, polypeptide, and/or hFGF-20 epitope recognizing antibody as described herein and a carrier or diluent. The carrier or diluent can optionally be pharmaceutically acceptable, according to known methods.

The present invention also provides a composition comprising an hFGF-20 polypeptide, hFGF-20 epitope recognizing antibody, and/or hFGF-20 nucleic acid wherein the composition has at least one activity including, but not limited to, neuroprotection, induction or inhibition of neurite outgrowth, induction or inhibition of neurite adhesion, induction of neural regeneration and/or neurorecuperation, inhibition of neural degeneration, inhibition or prevention of turmorigenesis, prevention or reduction in the frequency and/or severity of seizures, induction or inhibition of primary or secondary sexual development, or modulation of behavioral patterns including, but not limited to, sleep and eating disorders. A composition of the present invention can be evaluated for such activity according to these or any other methods known in the art. (Wagner, J. A., et al., J. of Cell Biol., 103(4):1363-7 (1986); Lein eta al., Neuron, 15:597-605 (1995); Neufeld, G., et al., J. of Cell. Phys., 131(1):131-40 (1987); Maiese, K., et al., J. of Neurosci., 13(7):3034-

40 (1993); Cheng, B., et al., Neuron, 7(6):1031-41 (1991); Fisher, M., et al., J. of Cereb. Blood Flow & Metabol., 15(6):953-9 (1995); Jiang, N., et al., J. Neuro. Sci., 139(2):173-9 (1996); Todo, T., et al., Brain Research, 783(2):179-87 (1998); Todo, T., et al., Neurosurgery, 43(2):337-46 (1998); Kawamata, T., et al., (1996); Kawamata, T., et al., (1997a); Kawamata, T., Speliotes, E. K., and Finklestein, S. P. Adv. in Neurol., 73:377-82, (1997b); Kolodkin, A., et al., Neuron, 21:1079-1092, (1998); Kolodkin, et al., (1997); Wilson et al., J. Cell Sci. 109:3129-3138 (1996); Pimenta et al., Neuron, 15:287-297 (1995), all of which are incorporated herein by reference).

Methods for the treatment or prevention of neuronal damage caused by disease, disorder, or trauma using the nucleic acids, polypeptides, antibodies, vectors, host cells, and/or transgenic cells described herein are also part of the invention. For instance, a method of treatment or prophylaxis for a nervous disease or disorder or a cancer can be effected with the polypeptides, nucleic acids, antibodies, vectors, host cells, transgenic cells, and/or compositions described herein. Accordingly, the present invention also includes methods for the prophylaxis or treatment of patho-physiological conditions in which at least one cell type involved in said condition is sensitive or responsive to a hFGF-20 polypeptide, hFGF-20 nucleic acid, hFGF-20 epitope recognizing antibody, hFGF-20 host cell, hFGF-20 transgenic cell, or hFGF-20 composition of the present invention.

The present invention also provides a method for identifying compounds that bind an hFGF-20 polypeptide, comprising

a) admixing at least one isolated hFGF-20 polypeptide as described herein with a test compound or composition; and

b) detecting at least one binding interaction between the polypeptide and the compound or composition, optionally further comprising detecting a change in biological activity, such as a reduction or increase.

The present invention also provides methods for identifying polypeptides that bind an hFGF-20 polypeptide which comprises use of at least one isolated hFGF-20 polypeptide as described herein in at least one protein-protein interaction assay or reporter system known in the art.

DESCRIPTION OF THE INVENTION

The invention provides human fibroblast growth factor 20 (hFGF-20) polypeptides, as well as nucleic acids encoding them and/or otherwise related thereto. The deduced amino acid sequence of hFGF-20 is 71% identical to that of human FGF-9. Human FGF-20 encoding polynucleotides and the human FGF-20 polypeptides encoded thereby were recently described in international patent application WO 00/54813 (the entire contents of which is incorporated herein by reference) and by Jeffers, M., et al., Cancer Res. 61(7):3131-8 (2001), and Ohmachi, S., et al., Biochem. Biophys. Res. Commun., 277(2):355-60 (2000) (both of which are entirely incorporated herein by reference).

The present invention provides isolated, recombinant and/or synthetic nucleic acid molecules comprising at least one polynucleotide encoding at least one hFGF-20 polypeptide comprising specific full length sequences, as well as fragments and/or specified variants thereof. An hFGF-20 polypeptide of the invention comprises at least one fragment, domain, and/or specified variant of an hFGF-20 polypeptide as shown in SEQ ID NO: 4.

The invention also provides methods for detecting alterations in FGF-20 gene expression, which can be used in

the diagnosis and prognosis of neurodegenerative and neoplastic disorders. Methods for treating neurodegenerative and neoplastic disorders, in which the expression and/or activity of an hFGF-20 is modulated, are also included in the invention.

The invention also relates to the therapy of human cancers which have a mutation in the hFGF-20 gene, including gene therapy, protein replacement therapy and protein mimetics.

Utility

Human FGF-20 encoding nucleic acids and the polypeptide encoded thereby are expressed in the developing and adult nervous systems, and play key roles in regulating nervous system development and function. Accordingly, hFGF-20 polypeptides and nucleic acids encoding them, in addition to molecules related thereto, can be used in methods for treating and/or diagnosing conditions affecting the nervous system (e.g., stroke, neurodegenerative diseases, such as Parkinson's disease and Alzheimer's disease, retinal degenerative diseases, such as retinitis pigmentosa and macular degeneration, and/or cerebellar degenerative diseases). Human FGF-20 nucleic acids, hFGF-20 polypeptides, as well as other molecules related thereto, can also be used to promote neuron growth *in vivo* or *in vitro*, in order to, for example, facilitate production of growth factors that are produced by them, such as interleukin-2 (IL-2). Additionally, hFGF-20 nucleic acids, hFGF-20 polypeptides, as well as other molecules related thereto, can also be used in methods for maintaining cultured cells or tissues, such as neuronal cells or tissues, prior to transplantation. Methods employing hFGF-20 polypeptides and nucleic acids are described in further detail below.

The chromosomal location of human FGF-20 at 8p21.3-p22 also implicates the human FGF-20 gene as a tumor suppressor gene. Accordingly, hFGF-20 nucleic acids and polypeptides may be used effectively to treat or prevent cancers including, but not limited to, hepatocellular, colorectal, testicular, and non-small cell lung cancer (Emi, M., et al., Genes, Chromosomes and Cancer, 7:152-157 (1993). The therapeutic agents of the present invention may take the form of polynucleotides comprising all or a portion of the FGF-20 locus placed in appropriate vectors or delivered to target cells in more direct ways such that the function of the FGF-20 protein is reconstituted. Therapeutic agents of the present invention may take the form of FGF-20 antisense polynucleotides placed in appropriate vectors and/or delivered to target cells such that the endogenous FGF-20 activity is inhibited. Therapeutic agents may also take the form of polypeptides based on either a portion of, or the entire protein sequence of hFGF-20. These polypeptides may functionally augment lost or diminished activity of endogenous FGF-20 *in vivo*. Therapeutic agents of the present invention may take the form of antibodies that bind to a FGF-20 polypeptide epitope that the endogenously expressed FGF-20 polypeptide activity is inhibited.

The present invention also provides at least one utility by providing isolated nucleic acid comprising polynucleotides of sufficient length and complementarity to an hFGF-20 nucleic acid for use as probes or amplification primers in the detection, quantitation, or isolation of gene sequences or transcripts. For example, isolated nucleic acids of the present invention can be used as probes for detecting deficiencies in the level of hFGF-20 mRNA or in screens for detection of genetic mutations in at least one hFGF-20 gene (e.g., substitutions, deletions, or additions).

Furthermore, isolated nucleic acids of the present

invention can be used monitoring regulation of expression of said gene or changes in biological activity as described herein in screening assays of compounds, and/or for detection of any number of allelic variants (polymorphisms or isoforms) of the gene.

The isolated nucleic acids of the present invention can also be used for recombinant expression of hFGF-20 polypeptides for use as immunogens in the preparation and/or screening of hFGF-20 recognizing antibodies. Monoclonal and polyclonal antibodies can be produced using standard immunization and screening methods well-known in the art. These antibodies can be easily and detectably labeled and used to identify tissues or biological samples which contain a hFGF-20 polypeptide.

The isolated nucleic acids of the present invention can also be employed for use in sense or antisense suppression of one or more hFGF-20 genes or nucleic acids, in a host cell, or tissue both *in vivo* and *in vitro*. Attachment of chemical agents which bind, intercalate, cleave and/or crosslink to the isolated nucleic acids of the present invention can also be used to modulate transcription or translation of at least one nucleic acid disclosed herein.

Citations

All publications or patents cited herein are entirely incorporated herein by reference as they show the state of the art at the time of the present invention to provide description and enablement of the present invention. Publications refer to scientific, patent publication or any other information available in any media format, including all recorded, electronic or printed formats. The following citations are entirely incorporated by reference: Ausubel, et al., eds., Current Protocols in Molecular Biology, John Wiley & Sons, N.Y., N.Y. (1987-1998); Coligan et al., eds.,

Current Protocols in Protein Science, John Wiley & Sons, Inc., N.Y., N.Y. (1995-1999); Sambrook, et al. (2001) Molecular Cloning: A Laboratory Manual, (3d ed.), vols. 1-3, CSH Press, NY (and, http://www.molecularcloning.com/); Harlow and Lane, Antibodies, a Laboratory Manual, Cold Spring Harbor, N.Y. (1989); Coligan, et al., eds., Current Protocols in Immunology, John Wiley & Sons, N.Y., N.Y. (1992-1999); Gennaro, Ed., Remington's Pharmaceutical Sciences, 18th Edition, Mack Publishing Co. (Easton, PA) 1990.

Definitions

The following definitions of terms are intended to correspond to those as well-known in the art. The following terms are therefore not limited to the definitions given, but are used according to the state of the art, as demonstrated by cited and/or contemporary publications or patents.

The term "activity" or the phrase "biological activity" in reference to a hFGF-20 polypeptide or hFGF-20 analog relates to the capacity of the particular hFGF-20 polypeptide or hFGF-20 analog to induce, *in vivo* and/or *in vitro,* biological consequences associated with such molecules and as discussed herein, including, but not limited to, neurotrophism, neurite outgrowth, and/or cellular proliferation. Accordingly, hFGF-20 polypeptide or hFGF-20 analog activity can be assessed by one or more of the *in vitro* or *in vivo* assays disclosed herein or otherwise known in the art.

The term "amino acid" is used herein in its broadest sense, and includes naturally occurring amino acids as well as non-naturally occurring amino acids, including amino acid analogs and derivatives. The latter includes molecules containing an amino acid moiety. One skilled in the art

will recognize, in view of this broad definition, that reference herein to an amino acid includes, for example, naturally occurring proteogenic L-amino acids; D-amino acids; chemically modified amino acids such as amino acid analogs and derivatives; naturally occurring non-proteogenic amino acids such as norleucine, β-alanine, ornithine, etc.; and chemically synthesized compounds having properties known in the art to be characteristic of amino acids.

The incorporation of non-natural amino acids, including synthetic non-native amino acids, substituted amino acids, or one or more D-amino acids into the hFGF-20 analogs of the present invention ("D- LP polypeptides") is advantageous in a number of different ways. D-amino acid-containing polypeptides exhibit increased stability *in vitro* or *in vivo* compared to L-amino acid-containing counterparts. Thus, the construction of polypeptides incorporating D-amino acids can be particularly useful when greater stability is desired or required *in vivo*. More specifically, D-peptides are resistant to endogenous peptidases and proteases, thereby providing improved bioavailability of the molecule, and prolonged lifetimes *in vivo* when such properties are desirable. When it is desirable to allow the peptide to remain active for only a short period of time, the use of L-amino acids therein will permit endogenous peptidases, proteases to digest the molecule, thereby limiting the cell's exposure to the molecule. Additionally, D-peptides cannot be processed efficiently for major histocompatibility complex class II-restricted presentation to T helper cells, and are therefore less likely to induce humoral immune responses in the whole organism.

In addition to using D-amino acids, those of ordinary skill in the art are aware that modifications in the amino acid sequence of a peptide, polypeptide, or protein can result in equivalent, or possibly improved, second

generation peptides that display equivalent or superior functional characteristics when compared to the original amino acid sequences. Alterations in the hFGF-20 analogs of the present invention can include one or more amino acid insertions, deletions, substitutions, truncations, fusions, shuffling of subunit sequences, and the like, either from natural mutations or human manipulation, provided that the sequences produced by such modifications have substantially the same (or improved or reduced, as may be desirable) activity(ies) as the hFGF-20 analog sequences disclosed herein. The term "hFGF-20 analog" as used herein refers to a modified form of a hFGF-20 polypeptide that exhibits substantially the same or even enhanced biological activity *in vivo* and/or *in vitro* as compared to an appropriate corresponding unmodified form of hFGF-20. However, preferred hFGF-20 analogs are those that are pharmaceutically more desirable, in at least one aspect, as compared to the appropriate corresponding unmodified hFGF-20 polypeptide. As used herein, the term "hFGF-20 analog" is intended to encompass hFGF-20 polypeptides as defined herein wherein the hFGF-20 polypeptide comprises at least one modification not typically native to the hFGF-20 polypeptide. The term "modification" includes any change in structure (ie., a qualitative change) of a protein. Such modifications can include, but are not limited to, changes in the amino acid sequence, transcriptional or translational splice variation, pre- or post- translational modifications to the DNA or RNA sequence, addition of macromolecules or small molecules to the DNA, RNA or protein, such as peptides, ions, vitamins, atoms, sugar-containing molecules, lipid-containing molecules, small molecules and the like, as well-known in the art. One type of protein modification according to the present invention is by one or more changes in the amino acid sequence (substitution, deltion or

insertion). Such changes can include, for example, at one or more amino acids, a change from a charged amino acid to a different charged amino acid, a non-charged to a charged amino acid, a charged amino acid to a non-charged amino acid as discussed, *infra.* or *supra.* Any other change in amino acid sequence is also included in the meaning of the term "analog". Another type of protein modification is by changes in processing of the protein in the cell. A non-limiting example is where some proteins have an "address label" specifying where in (in or outside of) the cell they should be used. Such a label or tag can be in the form of a peptide, a sugar or a lipid, which when added or removed from the protein, determines where the protein is located in the cell. A further type of protein modification arises from the attachment or removal of other macromolecules to a protein. These molecules can be of many types and can be either permanent or temporary. Preferred examples include: (i) polyribosylation, (ii) DNA/RNA (single or double stranded); (iii) lipids and phosphlipids (e.g., for membrane attachment); (iv) saccharides/polysaccharides; and (v) glycosylation (addition of different types of sugar and sialic acids -- in a variety of single and branched structures). Another type of protein modification is due to the attachment of other small molecules to proteins. Examples can include, but are not limited to: (i) phosphorylation; (ii) acetylation; (iii) uridylation; (iv) adenylation; (v) methylation, and (vi) capping (diverse complex modification of the N-terminus of the protein for assorted reasons). Most of these changes are often used to regulate a protein's activity. (v) and (vi) are also used to change the half-life of the protein itself. These protein changes can be detected on 2 dimensional gel electrophoresis incorporating several methods, such as labeling, changes in pI, antibodies or other specific techniques directed to the

molecules themselves, as known in the art. Molecular weight changes can be, but may not usually be detectable by 2DGE. MALD (matrix assisted laser desorption of flight mass spectrometry) is preferred to detect and characterize these modifications. Such modifications are generally aimed at improving upon a poor therapeutic characteristic of a native hFGF-20 polypeptide by increasing the molecule's target specificity, solubility, stability, serum half-life, affinity for targeted receptors, susceptibility to proteolysis, resistance to clearing *in vivo*, ease of purification, and/or decreasing the antigenicity and/or required frequency of administration.

In the present disclosure, the term "antibody" is intended to refer to a monoclonal antibody *per se*, or an immunologically effective fragment thereof. As is well known, antibodies are properly cross-linked via disulfide bonds. However, it is also known that the antigen-binding function of an antibody can be performed by fragments of a full-length antibody. Therefore, the term antibody without further limitation is intended to include phrases such as "antibody fragment(s)", "antibody portion(s)", "antigen binding portion(s)" or "fragment(s) thereof" in reference to an antibody refer to fragments of an antibody that retain the ability to specifically bind to a particular antigen. Examples of binding fragments encompassed within the term antibody include (i) a Fab fragment, a monovalent fragment consisting of the VL, VH, CL and CH1 domains; (ii) a F(ab')2 fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the VH and CH1 domains; (iv) a Fv fragment consisting of the VL and VH domains of a single arm of an antibody, (v) a dAb fragment (Ward *et al.*, *Nature*, 341:544-546 (1989)), which consists of a VH domain; and (vi) an isolated complementarity determining region (CDR).

Furthermore, although the two domains of the Fv fragment, VL and VH, are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein, chain in which the VL and VH regions pair to form monovalent molecules (known as single chain Fv(scFv): see e.g., Bird *et al.* (1988) *Science* 242:423-426; and Huston *et al.* (1988) *Proc. Natl. Acad. Sci.* USA 85:5879-5883). Also included within the definition "antibody" for example, are single chain forms, generally designated F_v regions, of antibodies. Other forms of single chain antibodies, such as diabodies are similarly encompassed with the definition of the term "antibody". Diabodies are bivalent, bispecific antibodies in which VH and VL domains are expressed on a single polypeptide chain, but using a linker that is too short to allow for pairing between the two domains on the same chain, thereby forcing the domains to pair with complementary domains of another chain and creating two antigen binding sites (see e.g., Hollinger P., *et al.* (1993) *Proc. Natl. Acad. Sci.* USA 90:6444-6448; Plijak, R.J., *et al.* (1994) *Structure* 2:1121-1123). Still further, an antibody, or more specifically, an antigen-binding portion thereof, may be part of a larger immunoadhesion molecule formed by covalent or non-covalent association of the antibody or antigen binding portion with one or more other proteins or peptides. Examples of such immunoadhesion molecules include use of the streptavidin core region to make a tetrameric scFv molecule (Kipriyanov, S.M., *et al* (1995) *Human Antibodies and Hybridomas* 6:93-101) and use of a cysteine residue, a marker peptide and a C-terminal polyhistidine tag to make bivalent and biotinylated scFv molecules (Kipriyanov, S.M., *et al* (1994) *Mol. Immunol.* 31:1047-1058). Antibody fragments, such as Fav and F(ab')2 fragments, can be prepared from whole antibodies using conventional techniques, such as

papain or pepsin digestion, respectively, of whole antibodies. Moreover, antibodies, antibody fragments, and immunoadhesion molecules can be obtained using standard recombinant DNA techniques as known in the art or as described herein. Unless stated otherwise, as long as the immunoglobulin protein demonstrates the ability to specifically bind its intended target, in this case, FGF-20 polypeptides, it is included within the term "antibody" as used herein. In particular, human and humanized antibodies are included within the meaning of the term "antibody".

CDR1, CDR2, or CDR3 of the heavy chain variable region alternatively are referred to hereinafter as H1, H2, and H3 respectively, and the CDR1, CDR2, and CDR3 of the light chain variable region are referred to hereinafter as L1, L2, and L3, respectively, of an antibody.

The term "human antibody" includes antibodies having variable and constant regions corresponding to human germline immunoglobulin sequences as described by *Kabat et al.* (1991). The human antibodies of the invention may include amino acid residues not encoded by human germline immunoglobulin sequences (e.g., mutations introduced by random or site-specific mutagenesis *in vitro* or by somatic mutation *in vivo*), for example in the CDRs and, in particular, CDR3. Any human antibody can also be substituted at one or more positions with an amino acid, e.g., a biological property enhancing amino acid residue, which is not encoded by the human germline immunoglobulin sequence. In preferred embodiments, these replacements are within the CDR regions as described in detail below.

Human antibodies have at least three advantages over non-human and chimeric antibodies for use in human therapy:

1) because the effector portion of the antibody is human, it may interact better with the other parts of the human immune system (e.g., destroy the target cells more

efficiently by complement-dependent cytotoxicity (CDC) or antibody-dependent cellular cytotoxicity (ADCC);

2) The human immune system should not recognize the human antibody as foreign, and therefore the antibody response against such an injected antibody should be less than against a totally foreign non-human antibody or a partially foreign chimeric antibody;

3) injected non-human antibodies have been reported to have a half-life in the human circulation much shorter than the half-life of human antibodies. Injected human antibodies will have a half-life essentially identical to naturally occurring human antibodies, allowing smaller and less frequent doses to be given.

The term "antagonist" in reference to a polypeptide is used in the broadest sense and includes any molecule that partially or fully blocks, inhibits, decreases, or neutralizes a biological activity of the polypeptide. Preferred antagonists of a polypeptide associated activity include neutralizing or antagonist antibodies. However, the term antagonist is also intended to include any molecule that partially or fully blocks, inhibits, decreases the expression of a polypeptide encoding polynucleotide or a polypeptide. As intended herein antagonists include nucleotide sequences, such as anti-sense and ribozyme molecules, and gene or regulatory sequence replacement constructs that can be used to inhibit expression of a messenger RNA transcript coding for a polypeptide. In a similar manner, the term "agonist" in reference to a polypeptide is used in the broadest sense and includes any molecule that induces or increases the expression of a polypeptide encoding polynucleotide or induces or increases the stability and/or biological activity of a polypeptide. An agonist may include for example, small molecules, naturally occurring ligand agonists, polypeptide ligand

agonists, and agonistic antibodies specific for an epitope of the polypeptide.

The terms "complementary" or "complementarity" as used herein refer to the capacity of purine, pyrimidine, synthetic or modified nucleotides to associate by partial or complete complementarity through hydrogen or other bonding to form partial or complete double- or triple-stranded nucleic acid molecules. The following base pairs occur by complete complementarity: (i) guanine (G) and cytosine (C); (ii) adenine (A) and thymine (T); and adenine (A) and uracil (U). "Partial complementarity" refers to association of two or more bases by one or more hydrogen bonds or attraction that is less than the complete complementarity as described above. Partial or complete complementarity can occur between any two nucleotides, including naturally occurring or modified bases, e.g., as listed in 37 CFR § 1.822. All such nucleotides are included in polynucleotides of the invention as described herein.

The term "fragment thereof" in reference to a hFGF-20 gene or cDNA sequence, refers to a fragment, or sub-region of an hFGF-20 nucleic acid such that said fragment comprises 10 or more nucleotides that are contiguous in the native nucleic acid molecule as shown in SEQ ID NO:1.

The term "fragment thereof" in reference to a hFGF-20 polypeptide or hFGF-20 analog refers to a fragment, or sub-region, of an hFGF-20 polypeptide or hFGF-20 analog such that said fragment comprises 5 or more amino acids that are contiguous in the native hFGF-20 polypeptide as shown in SEQ ID NO:4, or in a hFGF-20 analog, as the case may be.

"Functional fragment," as used herein, refers to an isolated sub-region, or fragment of a protein, or sequence of amino acids that, for example, comprises a functionally distinct region such as an active site on an enzyme, or a binding site for a ligand, receptor, polypeptide, or other

substrate. Functional fragments may be produced by recombinant DNA methodologies, enzymatic/proteolytic digestions, or as natural products of alternative splicing processes.

"Functionally-related" as used herein is applied to proteins or peptides that are functionally similar or identical to a particular protein or peptide. Molecules that would be expected to be functionally related to hFGF-20 polypeptides are those that are sufficiently homologous in their amino acid composition as compared with hFGF-20. For example, one or more conservative amino acid substitutions or deletions in the native hFGF-20 polypeptide as shown in SEQ ID NO:4 or 5, or in a specific hFGF-20 analog of the present invention, would not be expected to exhibit altered activity as compared to a native hFGF-20 polypeptide and, therefore, would be expected to be functionally related.

The term "hFGF-20" can refer to either a nucleic acid, gene, cDNA (e.g. SEQ ID NOS:1, 2, or 3), polypeptide (e.g., SEQ ID NOS:4 or 5) or any fragments, variants, analogs, or derivatives thereof. The term "hFGF-20" or "hFGF-20 polypeptide" without further limitation encompasses native human FGF-20 as shown in SEQ ID NO:4 as well as functional fragments thereof (including, but not limited to, the mature form of hFGF-20 polypeptide).

The term "fusion protein" denotes a hybrid protein molecule not found in nature comprising a translational fusion or enzymatic fusion in which two or more different proteins or fragments thereof are covalently linked on a single polypeptide chain. Known to one skilled in the art, is the fact that the human FGF-20 polypeptides of the present invention may be joined to other sequences (e.g., one or more pro- or pre-pro-sequences, processing of which will result in the production of a hFGF-20 polypeptide) and still retain a substantially similar or even greater

functional activity as compared to a corresponding hFGF-20 polypeptide of the present invention. The term "hFGF-20 polypeptide" is meant to encompass functional hFGF-20 polypeptides fusion proteins.

The phrase "hFGF-20 antibody" refers to an antibody as defined herein that recognizes and binds at least one epitope of a hFGF-20 polypeptide.

The term "FGF-20 allele" refers to normal alleles of the FGF-20 locus as well as alleles carrying variations that predispose individuals to develop cancer of many sites including, for example, melanoma, ocular melanoma, leukemia, astrocytoma, glioblastoma, lymphoma, glioma, Hodgkin's lymphoma, multiple myeloma, sarcoma, myosarcoma, cholangiocarcinoma, squamous cell carcinoma, CLL, and cancers of the pancreas, breast, brain, prostate, bladder, thyroid, ovary, uterus, testis, kidney, stomach, colon and rectum. Such predisposing alleles are also called "FGF-20 susceptibility alleles". "FGF-20 locus," "FGF-20 gene," "FGF-20 nucleic acids" or "FGF-20 polynucleotide" refer to polynucleotides, all of which are in the FGF-20 genetic region. Although likely to be expressed in normal tissue, certain alleles of the FGF-20 gene can predispose an individual to develop melanoma and other cancers, such as ocular melanoma, leukemia, astrocytoma, glioblastoma, lymphoma, glioma, Hodgkin's lymphoma, multiple myeloma, sarcoma, myosarcoma, cholangiocarcinoma, squamous cell carcinoma, CLL, and cancers of the pancreas, breast, brain, prostate, bladder, thyroid, lung, ovary, uterus, testis, kidney, stomach, colon and rectum. Mutations at the FGF-20 locus may also be involved in the initiation and/or progression of neurological disorders and/or other types of tumors. The locus is indicated in part by mutations that predispose individuals to develop at least one of the neurological disorders or cancers herein. The FGF-20 locus

is intended to include coding sequences, intervening sequences and regulatory elements controlling transcription and/or translation. The FGF-20 locus is intended to include all allelic variations of the DNA sequence. Specified deletional mutations as described herein are indicated by a "D" preceding the number of the amino acid deleted in that particular mutant. For example, D18 represents a mutant polypeptide which does not contain the eighteenth amino acid as compared to a designated comparator sequence.

The term "host cell" refers to any eucaryotic, procaryotic, or fusion or other cell or pseudo cell or membrane-containing construct that is suitable for propagating and/or expressing an isolated nucleic acid that is introduced into a host cell by any suitable means known in the art (e.g., but not limited to, transformation or transfection, or the like), or induced to express an endogenous nucleic acid encoding an hFGF-20 polypeptide according to the present invention. The cell can be part of a tissue or organism, isolated in culture or in any other suitable form.

The term "homolog" or "homologous" designates a relationship of partial identity or similarity of sequence between nucleic acid molecules or protein molecules at one or more regions within said molecules. As used herein, the term "sufficiently homologous" refers to a first amino acid or nucleotide sequence which contains a sufficient or minimum number of identical or or related amino acid substitutions (for related amino acids see Table 1 for conservative substitutions and discussion of groups, *infra*.) or nucleotides to a second amino acid or nucleotide sequence such that the first and second amino acid or nucleotide sequences have a common structural domain and/or common functionality. Preferably, a sufficiently homologous polypeptide comprises a defined region having at least about

85% homology, more preferably at least about 90% homology, more preferably at least about 95% homology, more preferably at least about 96% homology, more preferably at least about 97% homology, more preferably at least about 98% homology, more preferably at least about 99% homology, and most preferably 100% homology to the entire region as defined. Preferably, a sufficiently homologous polynucleotide comprises a polynucleotide extending over a defined length having at least about 85% homology, more preferably at least about 90% homology, more preferably at least about 95% homology, more preferably at least about 96% homology, more preferably at least about 97% homology, more preferably at least about 98% homology, more preferably at least about 99% homology, and most preferably 100% amino acid homology over the entire region as defined.

The term "hybridization" as used herein refers to a process in which a partially or completely single-stranded nucleic acid molecule joins with a complementary strand through nucleotide base pairing. Hybridization can occur under conditions of low, moderate or high stringency, with high stringency preferred. The degree of hybridization depends upon, for example, the degree of homology, the stringency conditions, and the length of hybridizing strands as known in the art.

The term "inhibit" or "inhibiting" includes the generally accepted meaning, which includes prohibiting, preventing, restraining, slowing, stopping, or reversing progression or severity of a disease or condition.

The terms "interacting polypeptide segment" and "interacting polypeptide sequence" refer to a portion of a hybrid protein which can form a specific binding interaction with a portion of a second hybrid protein under suitable binding conditions. Generally, a portion of the first hybrid protein preferentially binds to a portion of the

second hybrid protein forming a heterodimer or higher order heteromultimer comprising the first and second hybrid proteins; the binding portions of each hybrid protein are termed interacting polypeptide segments. Generally, interacting polypeptides can form heterodimers with a dissociation constant (K_D) of at least about 1 X 10^3 M^{-1}, usually at least 1 X 10^4 M^{-1}, typically at least 1 X 10^5 M^{-1}, preferably, at least 1 X 10^6 M^{-1} to 1 X 10^7 M^{-1}, or more, under suitable physiological conditions.

In the present disclosure, "isolated" refers to material removed from its original environment (e.g., the natural environment if it is naturally occurring), and thus is altered "by the hand of man" from its natural state. For example, the term "isolated" in reference to a polypeptide refers to a polypeptide that has been identified and separated and/or recovered from at least one contaminant from which it has been produced. Contaminants may include cellular components, such as enzymes, hormones, and other proteinaceous or non-proteinaceous solutes. Ordinarily, however, isolated polypeptides will be prepared by at least one purification step.

The term "isolated" in reference to a nucleic acid compound refers to any specific RNA or DNA molecule, however constructed or synthesized or isolated, which is locationally distinct from its natural location. For example, an isolated polynucleotide could be part of a vector or a composition of matter, or could be contained within a cell, and still be "isolated" because that vector, composition of matter, or particular cell is not the original environment of the polynucleotide.

An "isolated" antibody is one that has been identified and separated and/or recovered from a component of its natural environment. Contaminant components of its natural

environment are materials that would interfere with diagnostic or therapeutic uses for the antibody, and may include enzymes, hormones, and other proteinaceous or non-proteinaceous solutes. Ordinarily, an isolated antibody is prepared by at least one purification step. In preferred embodiments, the antibody will be purified (1) to greater than 95% by weight of antibody as determined by the Lowry method, and most preferably more than 99% by weight, (2) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a spinning cup sequenator, or (3) to homogeneity by SDS-PAGE under reducing or nonreducing conditions using Coomassie blue, or preferably, silver stain.

An "isolated antibody" is also intended to mean an antibody that is substantially purified from other antibodies having different antigenic specificities. An isolated antibody that specifically binds hFGF-20 epitopes may bind hFGF-20 homologous molecules from other species. The term "isolated" may be used interchangeably with the phrases "substantially pure" or "substantially purified" in reference to a macromolecule that is separated from other cellular and non-cellular molecules, including other proteins, lipids, carbohydrates or other materials with which it is naturally associated when produced recombinantly or synthesized without any general purifying steps. A "substantially pure" or "isolated" protein as described herein could be prepared by a variety of techniques well known to the skilled artisan. In preferred embodiments, a polypeptide will be isolated or substantialy purified upon purification (1) to greater than 85% by weight of polypeptide to the weight of total protein as determined by the Lowry method, and most preferably to more than 95% by weight of polypeptide to the weight of total protein, (2) to a degree sufficient to obtain at least 15 residues of N-

terminal or internal amino acid sequence by use of a spinning cup sequenator, or (3) to apparent homogeneity by SDS-PAGE under reducing or nonreducing conditions using Coomassie Blue, or preferably, silver stain, such that the major band constitutes at least 85%, and, more preferably 95%, of stained protein observed on the gel.

The term "mature protein" or "mature polypeptide" as used herein refers to the form(s) of the protein produced by expression in a mammalian cell. It is generally hypothesized that once export of a growing protein chain across the rough endoplasmic reticulum has been initiated, proteins secreted by mammalian cells have a signal peptide (SP) sequence which is cleaved from the complete polypeptide to produce a "mature" form of the protein. Oftentimes, cleavage of a secreted protein is not uniform and may result in more than one species of mature protein. The cleavage site of a secreted protein is determined by the primary amino acid sequence of the complete protein and generally can not be predicted with complete accuracy. Methods for predicting whether a protein has a SP sequence, as well as the cleavage point for that sequence, are available. Analysis of the amino acid sequence of the proteins described herein indicated that hFGF-20 did not have a typical signal sequence in its N terminus like those in acidic FGF and basic FGF. Although acidic FGF and basic FGF are known not to be secreted from cells in a conventional manner, rat FGF-9 was found to be secreted from cells after synthesis despite its lack of a typical signal sequence (Miyamoto, M., et al (1993)). It could be detected exclusively in the culture medium of cDNA-transfected COS cells. The amino acid sequence of proteins purified from culture supernatant of the CHO cell line, which was cDNA transfected and selected as a high producer of rat FGF-9, showed that no peptides were cleaved from the N terminus

except the initiation methionine. Therefore, a previously unknown but functional signal sequence may exist in the N-terminal domain of hFGF-20. A cleavage point may exist within the N-terminal domain between amino acid 10 and amino acid 35. More specifically the cleavage point is likely to exist after amino acid 15 but before amino acid 30, more likely after amino acid 20 but before amino acid 25, and most likely after amino acid 22 and before amino acid 23 as presented in SEQ ID NO:3. The resulting mature protein is represented in one non-limiting example by SEQ ID NO: 5. As one of ordinary skill would appreciate, however, cleavage sites sometimes vary from organism to organism and cannot be predicted with absolute certainty. Accordingly, the present invention provides polypeptides having a sequence of 90-100% of the contiguous sequence shown in SEQ ID NO: 5 which have an N-terminus beginning within 10 residues (i.e., + or - 10 residues) of the predicted cleavage point prior to the corresponding amino acid 23 as shown in SEQ ID NO:5. Optimally, cleavage sites for a secreted protein are determined experimentally by amino-terminal sequencing of the one or more species of mature proteins found within a purified preparation of the protein.

The term "multimer" comprises dimer and higher order complexes (trimer, tetramer, pentamer, hexamer, heptamer, octamer, etc.). "Homomultimer" refers to complexes comprised of the same subunit species. "Heteromultimer" refers to complexes comprised or more than one subunit species.

As used herein, the term "mutation" encompasses encompasses all forms of mutations including deletions, insertions and point mutations in the coding and noncoding regions. Deletions may be of the entire gene or only a portion of the gene. Point mutations may result in stop codons, frameshift mutations or amino acid substitutions.

Somatic mutations are those which occur only in certain tissues, e.g., in the tumor tissue, and are not inherited in the germline. Germline mutations can be found in any of a body's tissues and are inherited. If only a single allele is somatically mutated, an early neoplastic state is generally indicated. However, if both alleles are mutated, then a late neoplastic state is generally indicated. The term mutation encompasses all the above-listed types of differences from wild type nucleic acid sequence.

The term "neutralizing", "antagonizing", "antagonistic" in reference to an FGF-20 antibody or the phrase "antibody that antagonizes FGF-20 activity" is intended to refer to an antibody or antibody fragment whose binding to a FGF-20 epitope results in inhibition of a FGF-20 biological activity or a FGF-20 associated biological activity. Similarly, the term "agonistic" or "agonist" in reference to an FGF-20 antibody, small molecule, or naturally occurring FGF-20 ligand is intended to refer to one that enhances or stimulates FGF-20 associated biological activity. The effects of a putative FGF-20 agonist or a FGF-20 antagonist on FGF-20 activity or a FGF-20 associated activity can be assessed by measuring the effect of a putative FGF-20 agonist or a putative FGF-20 antagonist on one or more *in vitro* or *in vivo* indicators of FGF-20 activity.

The term "epitope tagged" where used herein refers to a chimeric polypeptide comprising a hFGF-20 polypeptide fused to a "epitope tag". The epitope tag has enough residues to provide an epitope against which an antibody may be made, or which can be identified by some other agent, yet is short enough such that it does not interfere with the activity of the hFGF-20 polypeptide. The eptitope tag preferably is also fairly unique so that the antibody does not substantially cross-react with other epitopes. Suitable tag polypeptides generally have at least six amino acid residues and usually

between about 8 to about 50 amino acid residues, or more preferably, between about 10 to about 20 residues.

The term "HIS tag" where used herein refers to a hFGF-20 polypeptide fused to a highly rich histidine polypeptide sequence. The HIS tag has enough histidine residues to provide a unique purification means to select for the properties of the repeated histidine residues, yet is short enough such that it does not interfere with the activity of the extracellular domain sequence of hFGF-20. Suitable tag polypeptides generally have at least six amino acid residues and usually between about 4 to about 20 amino acid residues (preferably, between about 4 to about 10 residues, and most preferably 6, such as HHHHHH). Several codons encoding a hemagglutinin fragment (i.e., an "HA" tag to facilitate purification) or HIS tag (see, e.g., Ausubel et al., ed., Current Protocols in Molecular Biology, John Wiley and Sons, NY (1987-1999)) followed by a termination codon and polyadenylation. The HA tag corresponds to an epitope derived from the influenza hemagglutinin polypeptide (Wilson et al., Cell 37:767-778 (1984)). The fusion of the HA tag to the target polypeptide allows easy detection and recovery of the recombinant polypeptide with an antibody that recognizes the HA epitope.

A "nucleic acid probe", "oligonucleotide probe", or "probe" as used herein comprises at least one detectably labeled or unlabeled nucleic acid which hybridizes under specified hybridization conditions with at least one other nucleic acid. This term also refers to a single- or partially double-stranded nucleic acid, oligonucleotide or polynucleotide that will associate with a complementary or partially complementary target nucleic acid to form at least a partially double-stranded nucleic acid molecule. A nucleic acid probe may be an oligonucleotide or a nucleotide polymer. A probe can optionally contain a detectable moiety

which may be attached to the end(s) of the probe or be internal to the sequence of the probe, termed a "detectable probe" or "detectable nucleic acid probe."

A "polynucleotide" comprises at least 10 nucleotides, preferably 20 nucleotides, more preferably 30 nucleotides, even more preferably, 40 to 100 nucleotides, or most preferably greater than 100 nucleotides of a nucleic acid (RNA, DNA or combination thereof), provided by any means, such as synthetic, recombinant isolation or purification method steps.

A "primer" is a nucleic acid fragment or oligonucleotide which functions as an initiating substrate for enzymatic or synthetic elongation of, for example, a nucleic acid molecule, e.g., using an amplification reaction, such as, but not limited to, a polymerase chain reaction (PCR), as known in the art.

The term "stringency" refers to hybridization conditions for nucleic acids in solution. High stringency conditions disfavor non-homologous base pairing. Low stringency conditions have much less of this effect. Stringency may be altered, for example, by changes in temperature and/or salt concentration, or other conditions, as well-known in the art.

A non-limiting example of "high stringency" conditions includes, for example, (a) a temperature of about 42°C, a formamide concentration of less than about 20%, and a low salt (SSC) concentration, (b) a temperature of about 65°C, or less, and a low salt (SSPE) concentration; or (c) hybridization in 0.5M NaHPO4, 7% sodium dodecyl sulfate (SDS), 1 mM EDTA at 65°C (See, e.g., Ausubel, et al., ed., Current Protocols in Molecular Biology, 1987-1998, Wiley Interscience, New York, at § 2.10.3). "SSC" comprises a hybridization and wash solution. A stock 20X SSC solution contains 3M sodium chloride, 0.3M sodium citrate, pH 7.0.

"SSPE" comprises a hybridization and wash solution. A 1X SSPE solution contains 180 mM NaCl, 9mM Na2HPO4, 0.9 mM NaH2PO4 and 1 mM EDTA, pH 7.4.

The term "neurite outgrowth" includes dendritic and axonal outgrowth from neuronal cells. One of skill in the art will be familiar with the term neurite. Human FGF-20 activity has been associated with dendritic outgrowth in the present disclosure, and therefore the term neurite or neuron growth or outgrowth is often used herein as being representative of hFGF-20 activity.

The term "vector" as used herein refers to a nucleic acid compound used for introducing exogenous or endogenous nucleic acid into host cells. A vector comprises a nucleotide sequence which may encode one or more polypeptide molecules. Plasmids, cosmids, viruses and bacteriophages, in a natural state or which have undergone recombinant engineering, are non-limiting examples of commonly used vectors to provide recombinant vectors comprising at least one desired isolated nucleic acid molecule.

The term "wild type" is applied to a standard or reference nucleotide sequence to which variations are compared. As defined, any variation from wild type is considered a mutation including naturally occurring sequence polymorphisms.

Pharmaceutical terms

The term "administer" or "administering" means to introduce by any means a therapeutic agent into the body of a mammal in order to prevent or treat a disease or condition.

"Chronic" administration refers to administration of the agent(s) in a continuous mode as opposed to an acute mode, so as to maintain the initial therapeutic effect (activity) for an extended period of time. "Intermittent"

administration is treatment that is not consecutively done without interruption, but rather is cyclic in nature.

Administration "in combination with" one or more further therapeutic agents includes simultaneous (concurrent) and consecutive administration in any order.

A "biologically-effective amount" is the minimal amount of a compound or agent that is necessary to impart a biological consequence to the extent that the biological consequence is measurable either directly or indirectly. Such determinations are routine and within the skill of an ordinarily skilled artisan.

A "therapeutically-effective amount" is the minimal amount of a compound or agent that is necessary to impart therapeutic benefit to a mammal. By administering graduated levels of a hFGF-20 polypeptide or hFGF-20 analog to a mammal in need thereof, a clinician skilled in the art can determine the therapeutically effective amount of the hFGF-20 polypeptide or hFGF-20 analog required for administration in order to treat or prevent the diseases, condition, disorders, and/or at least one symptom thereof, discussed herein. Such determinations are routine in the art and within the skill of an ordinarily skilled clinician.

"Carriers" as used herein include pharmaceutically acceptable carriers, excipients, or stabilizers which are nontoxic to the cell or mammal being exposed thereto at the dosages and concentrations employed. A "pharmaceutically acceptable carrier" refers to one that is non-toxic to recipients at the dosages and concentrations employed and is compatible with other ingredients of the formulation. For example, the formulation preferably does not include oxidizing agents and other compounds that are known to be deleterious to polypeptides. Often the pharmaceutically acceptable carrier is an aqueous pH buffered solution. Examples of pharmaceutically acceptable carriers include

buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid; low molecule weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immuno-globulins; hydrophilic polymers such as polyvinyl-pyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counterions such as sodium; and/or nonionic surfactants such as TWEEN®, polyethylene glycol (PEG), and PLURONICS™.

"Pharmaceutically acceptable salt" includes, but is not limited to, salts prepared with inorganic acids, such as chloride, sulfate, phosphate, diphosphate, hydrobromide, and nitrate salts, or salts prepared with an organic acid, such as malate, maleate, fumarate, tartrate, succinate, ethylsuccinate, citrate, acetate, lactate, methanesulfonate, benzoate, ascorbate, para-toluenesulfonate, palmoate, salicylate and stearate, as well as estolate, gluceptate and lactobionate salts. Similarly, salts containing pharmaceutically acceptable cations include, but are not limited to, sodium, potassium, calcium, aluminum, lithium, and ammonium (including substituted ammonium).

The term "mammal" as used herein refers to any mammal, including humans, domestic and farm animals, and zoo, sports or pet animals, such as cattle (e.g. cows), horses, dogs, sheep, pigs, rabbits, goats, cats, and non-domesticated animals like mice and rats. In a preferred embodiment of the present invention, the mammal being treated or administered to is a human or mouse.

A "small molecule" is defined herein to have a molecular weight below about 500 daltons.

The terms "treating", "treatment" and "therapy" as used

herein refer to curative therapy, prophylactic therapy, and preventative therapy. An example of "preventative therapy" is the prevention or lessening of a targeted disease or related condition thereto. Those in need of treatment include those already with the disease or condition as well as those prone to have the disease or condition to be prevented. The terms "treating", "treatment", and "therapy" as used herein also describe the management and care of a mammal for the purpose of combating a disease, or related condition, and includes the administration of hFGF-20 polypeptides or hFGF-20 analogs to alleviate the symptoms or complications of said disease, condition. Treating as used herein also includes the administration of the protein for cosmetic purposes.

All references herein to a disease, condition, or disorder are contemplated to encompass the other diseases, conditions, disorders, and/or symptoms generally associated with that particular disease, condition, or disorder by the medical community.

A "therapeutically-effective amount" is the minimal amount of a compound or agent that is necessary to impart therapeutic benefit to a mammal. By administering graduated levels of a hFGF-20 polypeptide or hFGF-20 analog to a mammal in need thereof, a clinician skilled in the art can determine the therapeutically effective amount of the hFGF-20 polypeptide or hFGF-20 analog in order to treat or prevent a particular disease condition, or disorder when it is administered, such as intravenously, subcutaneously, intraperitoneally, orally, or through inhalation. The precise amount of the compound required to be therapeutically effective will depend upon numerous factors, e.g., such as the specific binding activity of the compound, the delivery device employed, physical characteristics of the compound, purpose for the administration, in addition to

patient specific considerations. The amount of a compound that must be administered to be therapeutically effective are routine in the art and within the skill of an ordinarily skilled clinician.

Nucleic Acid Molecules

Using the information provided herein, such as the nucleotide sequences encoding at least 90-100% of the contiguous amino acids of at least one of SEQ ID NOS:4-5, specified fragments or variants thereof, or a deposited vector comprising at least one of these sequences, a nucleic acid molecule of the present invention encoding an hFGF-20 polypeptide can be obtained using well-known methods.

Nucleic acid molecules of the present invention can be in the form of RNA, such as mRNA, hnRNA, tRNA or any other form, or in the form of DNA, including, but not limited to, cDNA and genomic DNA obtained by cloning or produced synthetically, or any combination thereof. The DNA can be triple-stranded, double-stranded or single-stranded, or any combination thereof. Any portion of at least one strand of the DNA or RNA can be the coding strand, also known as the sense strand, or it can be the non-coding strand, also referred to as the anti-sense strand.

Isolated nucleic acid molecules of the present invention include nucleic acid molecules comprising an open reading frame (ORF) shown in at least one of SEQ ID NOS:1, 2, or 3; nucleic acid molecules comprising the coding sequence for an hFGF-20 polypeptide; and nucleic acid molecules which comprise a nucleotide sequence substantially different from those described above but which, due to the degeneracy of the genetic code, still encode at least one hFGF-20 polypeptide as described herein. Of course, the genetic code is well-known in the art. Thus, it would be routine for one skilled in the art to generate such

degenerate nucleic acid variants that code for specific hFGF-20 polypeptides of the present invention. See, e.g., Ausubel, et al., *supra*, and such nucleic acid variants are included in the present invention.

In a further embodiment, nucleic acid molecules are provided encoding the mature hFGF-20 polypeptide or the full-length hFGF-20 polypeptide lacking the N-terminal methionine. The invention also provides an isolated nucleic acid molecule having the nucleotide sequence shown in at least one of SEQ ID NOS:1, 2, 3, or the nucleotide sequence of the hFGF-20 cDNA contained in at least one of the above-described deposited clones listed herein, or a nucleic acid molecule having a sequence complementary thereto. Such isolated molecules, particularly nucleic acid molecules, are useful as probes for gene mapping by in situ hybridization with chromosomes, and for detecting transcription, translation, and/or expression of the hFGF-20 gene in human tissue, for instance, by Northern blot analysis for mRNA detection.

Unless otherwise indicated, all nucleotide sequences identified by sequencing a nucleic acid molecule herein can be or were identified using an automated nucleic acid sequencer. All amino acid sequences of polypeptides encoded by nucleic acid molecules identified herein can be or were identified by codon correspondence or by translation of a nucleic acid sequence identified using method steps as described herein or as known in the art. Therefore, as is well-known in the art, any nucleic acid sequence identified by this automated approach and identified herein may contain some errors which are reproducibly correctable by re-sequencing based upon an available or a deposited vector or host cell containing the nucleic acid molecule using well-known methods.

Nucleotide sequences identified by automation are typically at least about 95% to at least about 99.999% identical to the actual nucleotide sequence of the sequenced nucleic acid molecule. The actual sequence can be more precisely identified by other approaches including manual nucleic acid sequencing methods well-known in the art. As is also known in the art, a single insertion or deletion in an identified nucleotide sequence compared to the actual sequence will cause a frame shift in translation of the nucleotide sequence. As a result of the frame-shift the identified amino acid sequence encoded by an identified nucleotide sequence will be completely different from the amino acid sequence actually encoded by the sequenced nucleic acid molecule, beginning at the point of such an insertion or deletion.

Nucleic Acid Fragments

The present invention is further directed to fragments of the isolated nucleic acid molecules described herein. By a fragment of an isolated nucleic acid molecule it is meant a molecule having at least 10 nucleotides of a nucleotide sequence of a deposited cDNA or a nucleotide sequence shown in at least one of SEQ ID NOS:1, 2, or 3. It is further intended to mean fragments of at least about 10 nucleotides, and at least about 40 nucleotides in length, which are useful, *inter alia* as diagnostic probes and primers as described herein. Of course, larger fragments such as at least about 50, 100, 120, 200, 500, 1000, 1500, 2000, 2500, 3000, 3500, and/or 4000 or more nucleotides in length, are also useful according to the present invention as are fragments corresponding to most, if not all, of the nucleotide sequence (or the deposited cDNA) as shown at least one of SEQ ID NOS:1, 2, or 3. By a fragment at least 10 nucleotides in length, for example, is intended fragments

which include 10 or more contiguous nucleotides from the nucleotide sequence of the deposited cDNA or the nucleotide sequence as shown in at least one of SEQ ID NOS:1, 2, or 3 as determined by methods known in the art (See e.g., Ausubel, *supra*, Chapter 7).

Such nucleotide fragments are useful according to the present invention for screening DNA sequences that code for one or more fragments of an hFGF-20 polypeptide as described herein. Such screening, as a non-limiting example can include the use of so-called "DNA chips" for screening DNA sequences of the present invention of varying lengths, as described, e.g., in U.S. Patent Nos. 5,631,734, 5,624,711, 5,744,305, 5,770,456, 5,770,722, 5,675,443, 5,695,940, 5,710,000, 5,733,729, which are entirely incorporated herein by reference.

As indicated, nucleic acid molecules of the present invention can comprise a nucleic acid encoding an hFGF-20 polypeptide and can include, but is not limited to, those encoding the amino acid sequence of the mature polypeptide only. In addition, the present invention includes polynucleotides comprising the coding sequence for the mature polypeptide joined with additional coding sequences, such as the coding sequence of at least one signal leader or fusion peptide.

Also provided by the present invention are nucleic acid molecules encoding the mature hFGF-20 polypeptide, with or without the aforementioned additional coding sequences, together with additional, non-coding sequences, including but not limited to, introns and non-coding 5′ and 3′ sequences, such as any transcribed, non-translated sequences that play a role in transcription, mRNA processing, including splicing and polyadenylation signals (for example, ribosome binding and stability of mRNA). Furthermore, the present invention includes hFGF-20 polynucleotides encoding

hFGF-20 polypeptides having additional amino acids which provide additional functionalities. Thus, the sequence encoding a polypeptide can be fused to a marker sequence, such as a sequence encoding a peptide that facilitates purification of the fused polypeptide.

Preferred nucleic acid fragments of the present invention also include nucleic acid molecules encoding epitope-bearing portions of an hFGF-20 polypeptide.

Oligonucleotide and Polynucleotide Probes and/or Primers

In another aspect, the invention provides a polynucleotide (either DNA or RNA) that comprises at least about 20 nt, still more preferably at least about 30 nt, and even more preferably at least about 30-2000 nt of a nucleic acid molecule described herein. These are useful as diagnostic probes and primers as discussed above and in more detail below.

By a portion of a polynucleotide of "at least 10 nt in length," for example, is intended 10 or more contiguous nucleotides from the nucleotide sequence of the reference polynucleotide (e.g., at least one deposited nucleic acid or at least one nucleotide sequence as shown in at least one of SEQ ID NOS:1, 2, or 3).

Of course, a polynucleotide which hybridizes only to a poly-A sequence (such as the 3' terminal poly(A) of the hFGF-20 cDNA shown as SEQ ID NO:1, or to a complementary stretch of T (or U) resides, would not be included in a probe of the invention, since such a polynucleotide would hybridize to any nucleic acid molecule containing a poly (A) stretch or the complement thereof (e.g., practically any double-stranded cDNA clone).

The present invention also provides subsequences of full-length nucleic acids. Any number of subsequences can be obtained by reference to at least one of SEQ ID NOS:1, 2, 3,

or a complementary sequence thereof, and using primers which selectively amplify, under stringent conditions to: at least two sites to the polynucleotides of the present invention, or to two sites within the nucleic acid which flank and comprise a polynucleotide of the present invention, or to a site within a polynucleotide of the present invention and a site within the nucleic acid which comprises it. A variety of methods for obtaining 5' and/or 3' ends is well-known in the art. See, e.g., RACE (Rapid Amplification of Complementary Ends) as described in M. A. Frohman, PCR Protocols: A Guide to Methods and Applications, M. A. Innis, et al, Eds., Academic Press, Inc., San Diego, CA, pp. 28-38 (1990); see also, U.S. Patent No. 5,470,722, and Ausubel, et al., Current Protocols in Molecular Biology, Chapter 15, Eds., John Wiley & Sons, N.Y. (1989-1999). Thus, the present invention provides hFGF-20 polynucleotides having the sequence of the hFGF-20 gene, nuclear transcript, cDNA, or complementary sequences and/or subsequences thereof.

Primer sequences can be obtained by reference to a contiguous subsequence of a polynucleotide of the present invention. Primers are chosen to selectively hybridize, under PCR amplification conditions, to a polynucleotide of the present invention in an amplification mixture comprising a genomic and/or cDNA library from the same species. Generally, the primers are complementary to a subsequence of the amplified nucleic acid. In some embodiments, the primers will be constructed to anneal at their 5' terminal ends to the codon encoding the carboxy or amino terminal amino acid residue (or the complements thereof) of the polynucleotides of the present invention. The primer length in nucleotides is selected from the group of integers consisting of from at least 15 to 50. Thus, the primers can be at least 15, 18, 20, 25, 30, 40, or 50 nucleotides in length or any range or value therein. A non-annealing sequence at the 5' end of the

primer (a "tail") can be added, for example, to introduce a cloning site at the terminal ends of the amplified DNA.

The amplification primers may optionally be elongated in the 3' direction with additional contiguous or complementary nucleotides from the polynucleotide sequences from which they are derived. The number of nucleotides by which the primers can be elongated is selected from the group of integers consisting of from at least 1 to at least 25. Thus, for example, the primers can be elongated with an additional 1, 5, 10, or 15 nucleotides or any range or value therein. Those of skill will recognize that a lengthened primer sequence can be employed to increase specificity of binding (i.e., annealing) to a target sequence, or to add useful sequences, such as links or restriction sites (See e.g., Ausubel, *supra*, Chapter 15).

The amplification products can be translated using expression systems well-known to those of skill in the art and as discussed, infra. The resulting translation products can be confirmed as polypeptides of the present invention by, for example, assaying for the appropriate catalytic activity (e.g., specific activity and/or substrate specificity), or verifying the presence of one or more linear epitopes which are specific to a polypeptide of the present invention. Methods for protein synthesis from PCR derived templates are known in the art (See e.g., Ausubel, *supra*, Chapters 9, 10, 15; Coligan, Current Protocols in Protein Science, *supra*, Chapter 5) and available commercially. See, e.g., Amersham Life Sciences, Inc., Catalog '97, p. 354.

Polynucleotides Which Selectively Hybridize to a Polynucleotide as Described Herein

The present invention provides isolated nucleic acids that hybridize under selective hybridization conditions to a polynucleotide disclosed herein, e.g., SEQ ID NOS:1, 2, or 3.

Thus, the polynucleotides of this embodiment can be used for isolating, detecting, and/or quantifying nucleic acids comprising such polynucleotides. For example, polynucleotides of the present invention can be used to identify, isolate, or amplify partial or full-length clones in a deposited library. In some embodiments, the polynucleotides are genomic or cDNA sequences isolated, or otherwise complementary to, a cDNA from a human or mammalian nucleic acid library.

Preferably, the cDNA library comprises at least 80% full-length sequences, preferably at least 85% or 90% full-length sequences, and more preferably at least 95% full-length sequences. The cDNA libraries can be normalized to increase the representation of rare sequences. Low stringency hybridization conditions are typically, but not exclusively, employed with sequences having a reduced sequence identity relative to complementary sequences. Moderate and high stringency conditions can optionally be employed for sequences of greater identity. Low stringency conditions allow selective hybridization of sequences having about 70% sequence identity and can be employed to identify orthologous or paralogous sequences.

Optionally, polynucleotides of this invention will encode an epitope of a polypeptide encoded by the polynucleotides described herein. The polynucleotides of this invention embrace nucleic acid sequences that can be employed for selective hybridization to a polynucleotide encoding a polypeptide of the present invention.

Screening polypeptides for specific binding to antibodies or fragments can be conveniently achieved using peptide display libraries. This method involves the screening of large collections of peptides for individual members having the desired function or structure. Antibody screening of peptide display libraries is well-known in the

art. The displayed peptide sequences can be from 3 to 5000 or more amino acids in length, frequently from 5-100 amino acids long, and often from about 8 to 15 amino acids long. In addition to direct chemical synthetic methods for generating peptide libraries, several recombinant DNA methods have been described. One type involves the display of a peptide sequence on the surface of a bacteriophage or cell. Each bacteriophage or cell contains the nucleotide sequence encoding the particular displayed peptide sequence. Such methods are described in PCT Patent Publication Nos. 91/17271, 91/18980, 91/19818, and 93/08278. Other systems for generating libraries of peptides have aspects of both in vitro chemical synthesis and recombinant methods. See, PCT Patent Publication Nos. 92/05258, 92/14843, and 96/19256. See also, U.S. Patent Nos. 5,658,754; and 5,643,768. Peptide display libraries, vector, and screening kits are commercially available from such suppliers as Invitrogen (Carlsbad, CA).

Polynucleotides Complementary to the Polynucleotides

As indicated above, the present invention provides isolated nucleic acids comprising hFGF-20 polynucleotides, wherein the polynucleotides are complementary to the polynucleotides described herein, above. As those of skill in the art will recognize, complementary sequences base pair throughout the entirety of their length with such polynucleotides (i.e., have 100% sequence identity over their entire length). Complementary bases associate through hydrogen bonding in double-stranded nucleic acids. For example, the following base pairs are complementary: guanine and cytosine; adenine and thymine; and adenine and uracil. (See, e.g., Ausubel, *supra*, Chapter 67; or Sambrook, *supra*).

Construction of Nucleic Acids

The isolated nucleic acids of the present invention can be made using (a) standard recombinant methods, (b) synthetic techniques, (c) purification techniques, or combinations thereof, as well-known in the art.

The nucleic acids may conveniently comprise sequences in addition to a polynucleotide of the present invention. For example, a multi-cloning site comprising one or more endonuclease restriction sites may be inserted into the nucleic acid to aid in isolation of the polynucleotide. Also, translatable sequences may be inserted to aid in the isolation of the translated polynucleotide of the present invention. For example, a hexa-histidine marker sequence provides a convenient means to purify the proteins of the present invention. The nucleic acid of the present invention - excluding the polynucleotide sequence - is optionally a vector, adapter, or linker for cloning and/or expression of a polynucleotide of the present invention.

Additional sequences may be added to such cloning and/or expression sequences to optimize their function in cloning and/or expression, to aid in isolation of the polynucleotide, or to improve the introduction of the polynucleotide into a cell. Typically, the length of a nucleic acid of the present invention less the length of its polynucleotide of the present invention is less than 20 kilobase pairs, often less than 15 kb, and frequently less than 10 kb. Use of cloning vectors, expression vectors, adapters, and linkers is well-known in the art. (See, e.g., Ausubel, *supra*, Chapters 1-5; or Sambrook, *supra*).

Recombinant Methods for Constructing Nucleic Acids

The isolated nucleic acid compositions of this invention, such as RNA, cDNA, genomic DNA, or a hybrid

thereof, can be obtained from biological sources using any number of cloning methodologies known to those of skill in the art. In some embodiments, oligonucleotide probes that selectively hybridize, under stringent conditions, to the polynucleotides of the present invention are used to identify the desired sequence in a cDNA or genomic DNA library. While isolation of RNA, and construction of cDNA and genomic libraries is well-known to those of ordinary skill in the art. (See, e.g., Ausubel, *supra*, Chapters 1-7; or Sambrook, *supra*).

Nucleic Acid Screening and Isolation Methods

A cDNA or genomic library can be screened using a probe based upon the sequence of a polynucleotide of the present invention, such as those disclosed herein. Probes may be used to hybridize with genomic DNA or cDNA sequences to isolate homologous genes in the same or different organisms. Those of skill in the art will appreciate that various degrees of stringency of hybridization can be employed in the assay; and either the hybridization or the wash medium can be stringent. As the conditions for hybridization become more stringent, there must be a greater degree of complementarity between the probe and the target for duplex formation to occur. Temperature, ionic strength, pH and the presence of a partially denaturing solvent such as formamide can control the degree of stringency. Changing the polarity of the reactant solution through, for example, manipulation of the concentration of formamide within the range of 0% to 50% conveniently varies the stringency of hybridization. The degree of complementarity (sequence identity) required for detectable binding will vary in accordance with the stringency of the hybridization medium and/or wash medium. The degree of complementarity will optimally be 100%; however, it should be understood that minor sequence

variations in the probes and primers may be compensated for by reducing the stringency of the hybridization and/or wash medium.

Methods of amplification of RNA or DNA are well-known in the art and can be used according to the present invention without undue experimentation, based on the teaching and guidance presented herein.

Known methods of DNA or RNA amplification include, but are not limited to, polymerase chain reaction (PCR) and related amplification processes (see, e.g., U.S. Patent Nos. 4,683,195, 4,683,202, 4,800,159, 4,965,188, to Mullis, et al.; 4,795,699 and 4,921,794 to Tabor, et al; 5,142,033 to Innis; 5,122,464 to Wilson, et al.; 5,091,310 to Innis; 5,066,584 to Gyllensten, et al; 4,889,818 to Gelfand, et al; 4,994,370 to Silver, et al; 4,766,067 to Biswas; 4,656,134 to Ringold) and RNA mediated amplification which uses anti-sense RNA to the target sequence as a template for double-stranded DNA synthesis (U.S. Patent No. 5,130,238 to Malek, et al, with the tradename NASBA), the entire contents of which are herein incorporated by reference. (See, e.g., Ausubel, *supra,* Chapter 15; or Sambrook, *supra*)

For instance, polymerase chain reaction (PCR) technology can be used to amplify the sequences of polynucleotides of the present invention and related genes directly from genomic DNA or cDNA libraries. PCR and other in vitro amplification methods may also be useful, for example, to clone nucleic acid sequences that code for proteins to be expressed, to make nucleic acids to use as probes for detecting the presence of the desired mRNA in samples, for nucleic acid sequencing, or for other purposes. Examples of techniques sufficient to direct persons of skill through *in vitro* amplification methods are found in Berger, Sambrook, and Ausubel (e.g., Chapter 15) *supra*, as well as Mullis, et al., U.S. Patent No. 4,683,202 (1987); and Innis, et al., PCR

Protocols A Guide to Methods and Applications, Eds., Academic Press Inc., San Diego, CA (1990). Commercially available kits for genomic PCR amplification are known in the art. See, e.g., Advantage-GC Genomic PCR Kit (Clontech). The T4 gene 32 protein (Boehringer Mannheim) can be used to improve yield of long PCR products.

Synthetic Methods for Constructing Nucleic Acids

The isolated nucleic acids of the present invention can also be prepared by direct chemical synthesis by methods such as the phosphotriester method of Narang, et al., Meth. Enzymol. 68:90-99 (1979); the phosphodiester method of Brown, et al., Meth. Enzymol. 68:109-151 (1979); the diethylphosphoramidite method of Beaucage, et al., Tetra. Letts. 22:1859-1862 (1981); the solid phase phosphoramidite triester method described by Beaucage and Caruthers, Tetra. Letts. 22(20):1859-1862 (1981), e.g., using an automated synthesizer, e.g., as described in Needham-VanDevanter, et al., Nucleic Acids Res. 12:6159-6168 (1984); and the solid support method of U.S. Patent No. 4,458,066. Chemical synthesis generally produces a single-stranded oligonucleotide, which may be converted into double-stranded DNA by hybridization with a complementary sequence, or by polymerization with a DNA polymerase using the single strand as a template. One of skill in the art will recognize that while chemical synthesis of DNA can be limited to sequences of about 100 or more bases, longer sequences may be obtained by the ligation of shorter sequences.

Recombinant Expression Cassettes

The present invention further provides recombinant expression cassettes comprising a nucleic acid of the present invention. A nucleic acid sequence of the present invention, for example a cDNA or a genomic sequence encoding a full-

length polypeptide of the present invention, can be used to construct a recombinant expression cassette which can be introduced into at least one desired host cell. A recombinant expression cassette will typically comprise a polynucleotide of the present invention operably linked to transcriptional initiation regulatory sequences that will direct the transcription of the polynucleotide in the intended host cell.

Both heterologous and non-heterologous (i.e., endogenous) promoters can be employed to direct expression of the nucleic acids of the present invention. These promoters can also be used, for example, in recombinant expression cassettes to drive expression of antisense nucleic acids to reduce, increase, or alter hFGF-20 content and/or composition in a desired tissue.

In some embodiments, isolated nucleic acids which serve as promoter or enhancer elements can be introduced in the appropriate position (generally upstream) of a non-heterologous form of a polynucleotide of the present invention so as to up or down regulate expression of a polynucleotide of the present invention. For example, endogenous promoters can be altered *in vivo* or *in vitro* by mutation, deletion and/or substitution.

A polynucleotide of the present invention can be expressed in either sense or anti-sense orientation as desired. It will be appreciated that control of gene expression in either sense or anti-sense orientation can have a direct impact on the observable characteristics.

Another method of suppression is sense suppression. Introduction of nucleic acid configured in the sense orientation has been shown to be an effective means by which to block the transcription of target genes.

A variety of cross-linking agents, alkylating agents and radical generating species as pendant groups on

polynucleotides of the present invention can be used to bind, label, detect and/or cleave nucleic acids. Knorre, et al., Biochimie 67:785-789 (1985); Vlassov, et al., Nucleic Acids Res. 14:4065-4076 (1986); Iverson and Dervan, J. Am. Chem. Soc. 109:1241-1243 (1987); Meyer, et al., J. Am. Chem. Soc. 111:8517-8519 (1989); Lee, et al., Biochemistry 27:3197-3203 (1988); Home, et al., J. Am. Chem. Soc. 112:2435-2437 (1990); Webb and Matteucci, J. Am. Chem. Soc. 108:2764-2765 (1986); Nucleic Acids Res. 14:7661-7674 (1986); Feteritz, et al., J. Am. Chem. Soc. 113:4000 (1991). Various compounds to bind, detect, label, and/or cleave nucleic acids are known in the art. See, for example, U.S. Patent Nos. 5,543,507; 5,672,593; 5,484,908; 5,256,648; and 5,681941, each entirely incorporated herein by reference.

Vectors and Host Cells

The present invention also relates to vectors that include isolated nucleic acid molecules of the present invention, host cells that are harboring the recombinant vectors, and the production of hFGF-20 polypeptides or fragments and/or variants thereof by recombinant techniques, as is well-known in the art. See, e.g., Sambrook, et al., *supra*; Ausubel, *supra*, Chapters 1-9, each entirely incorporated herein by reference.

The polynucleotides can optionally be joined to a vector containing a selectable marker for propagation in a host. Generally, a plasmid vector is introduced in a precipitate, such as a calcium phosphate precipitate, or in a complex with a charged lipid. If the vector is a virus, it can be packaged in vitro using an appropriate packaging cell line and then transduced into host cells.

The DNA insert should be operatively linked to an appropriate promoter, such as the phage lambda PL promoter, the *E. coli lac*, *trp* and *tac* promoters, the SV40 early and

late promoters and promoters of retroviral LTRs, or any other suitable promoter. The skilled artisan will know other suitable promoters. The expression constructs will further contain sites for transcription initiation, termination and, in the transcribed region, a ribosome-binding site for translation. The coding portion of the mature transcripts expressed by the constructs will preferably include a translation initiating at the beginning and a termination codon (e.g., UAA, UGA or UAG) appropriately positioned at the end of the mRNA to be translated, with VAA and VAG preferred for mammalian or eukaryotic cell expression.

Expression vectors will preferably include at least one selectable marker. Such markers include, e.g., dihydrofolate reductase, ampicillin (G418), hygromycin or neomycin resistance for eukaryotic cell culture, and tetracycline or ampicillin resistance genes for culturing in E. coli and other bacteria or prokaryotics. Representative examples of appropriate hosts include, but are not limited to, bacterial cells, such as *E. coli*, *Streptomyces* and *Salmonella typhimurium* cells; fungal cells, such as yeast cells; insect cells such as Drosophila S2 and Spodoptera Sf9 cells; animal cells such as CHO, COS and Bowes melanoma cells; and plant cells. Appropriate culture mediums and conditions for the above-described host cells are known in the art. Vectors preferred for use in bacteria include pQE70, pQE60 and pQE-9, available from Qiagen; pBS vectors, Phagescript vectors, Bluescript vectors, pNH8A, pNH16a, pNH18A, pNH46A, available from Stratagene; and ptrc99a, pKK223-3, pKK233-3, pDR540, pRIT5 available from Pharmacia. Preferred eucaryotic vectors include pWLNEO, pSV2CAT, pOG44, pXT1 and pSG available from Stratagene; and pSVK3, pBPV, pMSG and pSVL available from Pharmacia. Other suitable vectors will be readily apparent to the skilled

artisan. See, e.g., Ausubel, *supra*, Chapter 1; Coligan, Current Protocols in Protein Science, *supra*, Chapter 5.

Introduction of a vector construct into a host cell can be effected by calcium phosphate transfection, DEAE-dextran mediated transfection, cationic lipid-mediated transfection, electroporation, transduction, infection or other methods. Such methods are described in many standard laboratory manuals, such as Sambrook, *supra*, Chapters 1-4 and 16-18; Ausubel, *supra*, Chapters 1, 9, 13, 15, 16.

Polypeptide(s) of the present invention can be expressed in a modified form, such as a fusion protein, and can include not only secretion signals, but also additional heterologous functional regions. For instance, a region of additional amino acids, particularly charged amino acids, can be added to the N-terminus of a polypeptide to improve stability and persistence in the host cell, during purification, or during subsequent handling and storage. Also, peptide moieties can be added to a polypeptide to facilitate purification. Such regions can be removed prior to final preparation of a polypeptide. Such methods are described in many standard laboratory manuals, such as Sambrook, *supra*, Chapters 17 and 18; Ausubel, *supra*, Chapters 16, 17 and 18.

Expression of Proteins in Host Cells

Using nucleic acids of the present invention, one may express a protein of the present invention in a recombinantly engineered cell, such as bacteria, yeast, insect, or mammalian cells. The cells produce the protein in a non-natural condition (e.g., in quantity, composition, location, and/or time), because they have been genetically altered through human intervention to do so.

It is expected that those of skill in the art are knowledgeable in the numerous expression systems available

for expression of a nucleic acid encoding a protein of the present invention. No attempt to describe in detail the various methods known for the expression of proteins in prokaryotes or eukaryotes will be made.

In brief summary, the expression of isolated nucleic acids encoding a protein of the present invention will typically be achieved by operably linking, for example, the DNA or cDNA to a promoter (which is either constitutive or inducible) followed by incorporation into an expression vector. The vectors can be suitable for replication and integration in either prokaryotes or eukaryotes. Typical expression vectors contain transcription and translation terminators, initiation sequences and promoters useful for regulation of the expression of the DNA encoding a protein of the present invention. To obtain high level expression of a cloned gene, it is desirable to construct expression vectors which contain, at the minimum, a strong promoter to direct transcription, a ribosome binding site for translational initiation, and a transcription/translation terminator. One of skill would recognize that modifications can be made to a protein of the present invention without diminishing its biological activity. Some modifications may be made to facilitate the cloning, expression, or incorporation of the targeting molecule into a fusion protein. Such modifications are well-known to those of skill in the art and include, for example, a methionine added at the amino terminus to provide an initiation site, or additional amino acids (e.g., poly His) placed on either terminus to create conveniently located restriction sites or termination codons or purification sequences.

Alternatively, nucleic acids of the present invention can be expressed in a host cell by turning on (by manipulation) in a host cell that contains endogenous DNA encoding a polypeptide of the present invention. Such

methods are well-known in the art, e.g., as described in US patent Nos. 5,580,734, 5,641,670, 5,733,746, and 5,733,761, entirely incorporated herein by reference.

Expression in Prokaryotes

Prokaryotic cells may be used as hosts for expression. Prokaryotes most frequently are represented by various strains of E. coli; however, other microbial strains may also be used. Commonly used prokaryotic control sequences which are defined herein to include promoters for transcription initiation, optionally with an operator, along with ribosome binding site sequences, include such commonly used promoters as the beta lactamase (penicillinase) and lactose (lac) promoter systems (Chang, et al., Nature 198:1056 (1977)), the tryptophan (trp) promoter system (Goeddel, et al., Nucleic Acids Res. 8:4057 (1980)) and the lambda derived P L promoter and N-gene ribosome binding site (Shimatake, et al., Nature 292:128 (1981)). The inclusion of selection markers in DNA vectors transfected in E. coli is also useful. Examples of such markers include genes specifying resistance to ampicillin, tetracycline, or chloramphenicol.

The vector is selected to allow introduction into the appropriate host cell. Bacterial vectors are typically of plasmid or phage origin. Appropriate bacterial cells are infected with phage vector particles or transfected with naked phage vector DNA. If a plasmid vector is used, the bacterial cells are transformed with the plasmid vector DNA. Expression systems for expressing a protein of the present invention are available using Bacillus sp. and Salmonella (Palva, et al., Gene 22:229-235 (1983); Mosbach, et al., Nature 302:543-545 (1983)). See, e.g., Ausubel, *supra*, Chapters 1-3, 16(Sec.1); and Coligan, *supra*, Current Protocols in Protein Science, Units 5.1, 6.1-6.7).

Expression in Eukaryotes

A variety of eukaryotic expression systems such as yeast, insect cell lines, plant and mammalian cells, are known to those of skill in the art. As explained briefly below, a nucleic acid of the present invention can be expressed in these eukaryotic systems.

Synthesis of heterologous proteins in yeast is well-known. F. Sherman, et al., Methods in Yeast Genetics, Cold Spring Harbor Laboratory (1982) is a well-recognized work describing the various methods available to produce the protein in yeast. Two widely utilized yeast for production of eukaryotic proteins are Saccharomyces cerevisiae and Pichia pastoris. Vectors, strains, and protocols for expression in Saccharomyces and Pichia are known in the art and available from commercial suppliers (e.g., Invitrogen). Suitable vectors usually have expression control sequences, such as promoters, including 3-phosphoglycerate kinase or alcohol oxidase, and an origin of replication, termination sequences and the like as desired.

A protein of the present invention, once expressed, can be isolated from yeast by lysing the cells and applying standard protein isolation techniques to the lysates. The monitoring of the purification process can be accomplished by using Western blot techniques or radioimmunoassay of other standard immunoassay techniques.

The sequences encoding proteins of the present invention can also be ligated to various expression vectors for use in transfecting cell cultures of, for instance, mammalian, insect, or plant origin. Illustrative of cell cultures useful for the production of the peptides are mammalian cells. Mammalian cell systems often will be in the form of monolayers of cells although mammalian cell suspensions may also be used. A number of suitable host cell lines capable of expressing intact proteins have been developed in the art,

and include the HEK293, BHK21, and CHO cell lines. Expression vectors for these cells can include expression control sequences, such as an origin of replication, a promoter (e.g., the CMV promoter, a HSV tk promoter or pgk (phosphoglycerate kinase) promoter), an enhancer (Queen, et al., Immunol. Rev. 89:49 (1986)), and processing information sites, such as ribosome binding sites, RNA splice sites, polyadenylation sites (e.g., an SV40 large T Ag poly A addition site), and transcriptional terminator sequences. Other animal cells useful for production of proteins of the present invention are available, for instance, from the American Type Culture Collection Catalogue of Cell Lines and Hybridomas (7th edition, 1992).

Appropriate vectors for expressing proteins of the present invention in insect cells are usually derived from the SF9 baculovirus. Suitable insect cell lines include mosquito larvae, silkworm, armyworm, moth and Drosophila cell lines such as a Schneider cell line (See Schneider, J. Embryol. Exp. Morphol. 27:353-365 (1987).

As with yeast, when higher animal or plant host cells are employed, polyadenlyation or transcription terminator sequences are typically incorporated into the vector. An example of a terminator sequence is the polyadenlyation sequence from the bovine growth hormone gene. Sequences for accurate splicing of the transcript may also be included. An example of a splicing sequence is the VP1 intron from SV40 (Sprague, et al., J. Virol. 45:773-781 (1983)). Additionally, gene sequences to control replication in the host cell may be incorporated into the vector such as those found in bovine papilloma virus type-vectors. (M. Saveria-Campo, Bovine Papilloma Virus DNA, a Eukaryotic Cloning Vector in DNA Cloning Vol. II, a Practical Approach, D. M. Glover, Ed., IRL Press, Arlington, VA, pp. 213-238 (1985)).

Protein Purification

An hFGF-20 polypeptide can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Most preferably, high performance liquid chromatography ("HPLC") is employed for purification. Polypeptides of the present invention include naturally purified products, products of chemical synthetic procedures, and products produced by recombinant techniques from a prokaryotic or eucaryotic host, including, for example, bacterial, yeast, higher plant, insect and mammalian cells. Depending upon the host employed in a recombinant production procedure, the polypeptides of the present invention can be glycosylated or can be non-glycosylated. In addition, polypeptides of the invention can also include an initial modified methionine residue, in some cases as a result of host-mediated processes. Such methods are described in many standard laboratory manuals, such as Sambrook, supra, Chapters 17.37-17.42; Ausubel, supra, Chapters 10, 12, 13, 16, 18 and 20.

Human FGF-20 Polypeptides and Fragments and/or Variants Thereof

The invention further provides an isolated hFGF-20 polypeptide comprising a hFGF-20 polypeptide having an amino acid sequence as encoded by the deposited cDNAs or as shown in SEQ ID NOS:4 or 5, as well as any fragment and/or specified variant thereof. Exemplary polypeptide sequences are provided in SEQ ID NOS:4-5. The polypeptides of the present invention can comprise any number of contiguous amino acid residues from a polypeptide of the present invention,

wherein that number is selected from the group of integers consisting of from 90-100% of the number of contiguous residues in a hFGF-20 polypeptide. Optionally, this subsequence of contiguous amino acids is at least 50, 60, 70, 80, or 90 amino acids in length. Further, the number of such subsequences can be any integer selected from the group consisting of from 1 to 20, such as 2, 3, 4, or 5.

As those of skill will appreciate, the present invention includes biologically active polypeptides of the present invention (i.e., enzymes). Biologically active polypeptides have a specific activity at least 20%, 30%, or 40%, and preferably at least 50%, 60%, or 70%, and most preferably at least 80%, 90%, or 95%-1000% of that of the native (non-synthetic), endogenous polypeptide. Further, the substrate specificity (e.g., k_{cat}/K_m) is optionally substantially similar to the native (non-synthetic), endogenous polypeptide. Typically, the K_m will be at least 30%, 40%, or 50%, that of the native (non-synthetic), endogenous polypeptide; and more preferably at least 60%, 70%, 80%, or 90%-1000%. Methods of assaying and quantifying measures of enzymatic activity and substrate specificity, are well-known to those of skill in the art.

Generally, the polypeptides of the present invention will, when presented as an immunogen, elicit production of an antibody specifically reactive to a polypeptide of the present invention encoded by a polynucleotide of the present invention as described, supra. Exemplary polypeptides include those which are full-length, such as those disclosed herein. However, polypeptides of the invention also include fragments of FGF-20 polypeptides such as that retain at least one FGF-20-specific activity or epitope. For example, an FGF-20 polypeptide fragment containing, e.g., at least 8-10 amino acids can be used as an immunogen in the production of FGF-20-specific antibodies. The fragment can contain, for

example, an amino acid sequence that is conserved in other FGF-20 polypeptides. In addition to their use as peptide immunogens, the above-described FGF-20 fragments can be used in immunoassays, such as ELISAs, to detect the presence of FGF-20-specific antibodies in samples. Further, the proteins of the present invention will not bind to antisera raised against a polypeptide of the present invention that has been fully immunosorbed with the same polypeptide. Immunoassays for determining binding are well-known to those of skill in the art. A preferred immunoassay is a competitive immunoassay as discussed, infra. Thus, the proteins of the present invention can be employed as immunogens for constructing antibodies immunoreactive to a protein of the present invention for such exemplary utilities as immunoassays or protein purification techniques.

An hFGF-20 polypeptide of the present invention can include one or more amino acid substitutions, deletions or additions, either from natural mutations or human manipulation, as specified herein.

Of course, the number of amino acid substitutions a skilled artisan would make depends on many factors, including those described above. Generally speaking, the number of amino acid substitutions, insertions or deletions for any given hFGF-20 polypeptide will not be more than 40, 30, 20, 10, 5, or 3, such as 1-30 or any range or value therein, as specified herein.

Amino acids in an hFGF-20 polypeptide of the present invention that are essential for protein-protein binding or ligand-protein binding can be identified by methods known in the art, such as site-directed mutagenesis or alanine-scanning mutagenesis (Cunningham and Wells, Science 244:1081-1085 (1989)). The latter procedure introduces single alanine mutations at every residue in the molecule. The resulting mutant molecules are then tested for

biological activity. Sites that are critical for protein-protein binding or ligand-protein binding can also be identified by structural analysis such as crystallization, nuclear magnetic resonance or photoaffinity labeling (Smith, et al., J. Mol. Biol. 224:899-904 (1992) and de Vos, et al., Science 255:306-312 (1992)).

A hFGF-20 polypeptide of the present invention can include, but is not limited to, the mature protein form of the full-length hFGF-20 polypeptide as shown in SEQ ID NOS:5.

An hFGF-20 polypeptide can also include an amino acid sequence selected from SEQ ID NOS: 4 or 5. Non-limiting mutants that can enhance or maintain at least one of the biological activities disclosed herein include, but are not limited to, any of the above hFGF-20 polypeptides, further comprising at least one mutation corresponding to at least one substitution, insertion, or deletion selected from the group consisting of 5G, 9N, 10Y, 11F, 13V, 14Q, 15D, 16A, 17V, 18, 19, 20P, 21F, 23N, 24V, 25P, 26V, 29V, 50R, 53A, 54V, 55T, 56D, 58D, 61K, 78E, 80F, 82N, 84T, 85I, 90K, 94R, 103T, 123E, 135Q, 138V, 155L, 159V, 166Y, 177E, 179T, 181T, 198D, 199K, 207I, 209S, 210Q, and 211S of SEQ ID NO:4 or the corresponding amino acid of SEQ ID NO:5.

Antigenic/Epitope Comprising hFGF-20 Peptide and Polypeptides

In another aspect, the invention provides a peptide or polypeptide comprising an epitope-bearing portion of a polypeptide of the invention according to methods well-known in the art. See, e.g., Colligan, ed., Current Protocols in Immunology, Greene Publishing, NY (1993-1998), Ausubel, *supra*, each entirely incorporated herein by reference.

The epitope of this polypeptide portion is an immunogenic or antigenic epitope of a polypeptide described

herein. An "immunogenic epitope" can be defined as a part of a polypeptide that elicits an antibody response when the whole polypeptide is the immunogen. On the other hand, a region of a polypeptide molecule to which an antibody can bind is defined as an "antigenic epitope." The number of immunogenic epitopes of a polypeptide generally is less than the number of antigenic epitopes. See, for instance, Geysen, et al., Proc. Natl. Acad. Sci. USA 81:3998-4002 (1983).

As to the selection of peptides or polypeptides bearing an antigenic epitope (i.e., that contain at least a portion of a region of a polypeptide molecule to which an antibody can bind), it is well-known in the art that relatively short synthetic peptides that mimic part of a polypeptide sequence are routinely capable of eliciting an antiserum that reacts with the partially mimicked polypeptide. See, for instance, J. G. Sutcliffe, et al., "Antibodies that react with preidentified sites on polypeptides," Science 219:660-666 (1983).

Antigenic epitope-bearing peptides and polypeptides of the invention are useful to raise antibodies, including monoclonal antibodies, or screen antibodies, including fragments or single chain antibodies, that bind specifically to a polypeptide of the invention. See, e.g., Wilson, et al., Cell 37:767-778 (1984) at 777. Antigenic epitope-bearing peptides and polypeptides of the invention preferably contain a sequence of at least five, more preferably at least nine, and most preferably between at least about 15 to about 30 amino acids contained within the amino acid sequence of a polypeptide of the invention.

The epitope-bearing peptides and polypeptides of the invention can be produced by any conventional means. R. A. Houghten, "General method for the rapid solid-phase synthesis of large numbers of peptides: specificity of

antigen-antibody interaction at the level of individual amino acids," Proc. Natl. Acad. Sci. USA 82:5131-5135 (1985). This "Simultaneous Multiple Peptide Synthesis (SMPS)" process is further described in U.S. Patent No. 4,631,211 to Houghten, et al. (1986).

As one of skill in the art will appreciate, hFGF-20 polypeptides of the present invention and the epitope-bearing fragments thereof described above can be combined with parts of the constant domain of immunoglobulins (IgG), resulting in chimeric polypeptides. These fusion proteins facilitate purification and show an increased half-life in vivo. This has been shown, e.g., for chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins (EPA 394,827; Traunecker, et al., Nature 331:84-86 (1988)). Fusion proteins that have a disulfide-linked dimeric structure due to the IgG part can also be more efficient in binding and neutralizing other molecules than the monomeric hFGF-20 polypeptide or polypeptide fragment alone (Fountoulakis, et al., J. Biochem. 270:3958-3964 (1995)).

Production of Antibodies

The polypeptides of this invention and fragments and/or variants thereof may be used in the production of antibodies. These antibodies can be made by using an FGF-20 polypeptide, or an FGF-20 polypeptide fragment that maintains an FGF-20 epitope, as an immunogen in standard antibody production methods (see, e.g., Kohler, et al., Nature, 256:495, 1975; Ausubel, et al., supra; Harlow and Lane, supra).

Polyclonal antibodies are heterogeneous populations of antibody molecules derived from the sera of animals immunised with an antigen while monoclonal antibodies (MAbs) are

a substantially homogeneous population of antibodies to specific antigens. Polyclonal and MAbs may be obtained by methods known to those skilled in the art (for MAbs, see, for example, Kohler et al., *Nature 256:495-497* (1975), Colligan, supra., and U.S. Pat. No. 4,376,110).

Single chain antibodies and libraries thereof are yet another variety of genetically engineered antibody technology that is well-known in the art. (See, e.g., R. E. Bird, et al., Science 242:423-426 (1988); PCT Publication Nos. WO 88/01649, WO 90/14430, and WO 91/10737. Single chain antibody technology involves covalently joining the binding regions of heavy and light chains to generate a single polypeptide chain. The binding specificity of the intact antibody molecule is thereby reproduced on a single polypeptide chain.

MAbs may be of any immuno-globulin class including IgG, IgM, IgE, IgA, IgD and any subclass thereof. The hybridoma producing the MAbs of this invention may be cultivated *in vitro* or *in vivo*. Production of high titers of MAbs *in vivo* makes this the presently preferred method of production. Briefly, cells from the individual hybridomas are injected intraperitoneally into pristane-primed BALB/C mice to produce ascites fluid containing high concentrations of the desired MAbs. MAbs of isotype IgM or IgG may be purified from such ascites fluids, or from culture supernatants, using column chromatography methods well-known to those of skill in the art.

Chimeric antibodies are molecules in which different portions are derived from different animal species, such as those having variable region derived from a murine MAb and a human immunoglobulin constant region. Chimeric antibodies and methods for their production are known in the art (Cabilly et al., *Proc. Natl. Acad. Sci. (USA)* 71:3273-3277 (1984); Morrison et al., *Proc. Natl. Acad. Sci. (USA)*

81:6851-6855 (1984); Boulianne et al., *Nature* 312:643646 (1984); Cabilly et al., European Patent Application 125023 (published Nov. 14, 1984); Neuberger et al., *Nature* 314:268-270 (1985); Taniguchi et al., European Patent Application 171496 (published Feb. 19, 1985); Morrison et al., European Patent Application 173494 (published Mar. 5, 1986); Neuberger et al., PCT Application WO 86/01533 (published Mar. 13, 1986); Kudo et al., European Patent Application 184187 (published Jun. 11, 1986); Sahagan et al., *J. Immunol.* 137:1066-1074 (1986); Robinson et al., International Patent Publication #PCT/US86/02269 (published May 7,1987); Liu et al., *Proc. Natl. Acad. Sci. (USA) 84:3439-3443* (1987); Sun et al., *Proc. Natl. Acad. Sci. (USA)* 84:214-218 (1987); Better et al., *Science* 140:1041-1043 (1988)). These documents are hereby incorporated by reference.

The most preferred method of generating MAbs to the polypeptides and glycopeptides of the present invention comprises producing said MAbs in a transgenic mammal modified in such a way that they are capable of producing fully humanized MAbs upon antigenic challenge. Fully humanized MAbs and methods for their production are generally known in the art (PCT/WO9634096, PCT/WO9633735, and PCT/WO9824893). These documents are hereby incorporated by reference.

An anti-idiotypic (anti-Id) antibody is an antibody which recognizes unique determinants generally associated with the antigen-binding site of an antibody. An anti-Id antibody can be prepared by immunizing an animal of the same species and genetic type (e.g. mouse strain) as the source of the MAb with the MAb to which an anti-Id is being prepared. The immunized animal will recognize and respond to the idiotypic determinants of the immunizing antibody by producing an antibody to these idiotypic determinants (the

anti-Id antibody). The anti-Id antibody may also be used as an "immunogen" to induce an immune response in yet another animal, producing a so-called anti-anti-Id antibody. The anti-anti-Id may be epitopically identical to the original MAb which induced the anti-Id. Thus, by using antibodies to the idiotypic determinants of a MAb, it is possible to identify other clones expressing antibodies of identical specificity. Accordingly, MAbs generated against a hFGF-20 protein or glycoprotein of the present invention may be used to induce anti-Id antibodies in suitable animals, such as BALB/C mice and/or any transgenically altered mouse capable of producing fully humanized MAbs. Spleen cells from such immunized mice are used produce anti-Id hybridomas secreting anti-Id MAbs. Further, the anti-Id MAbs can be coupled to a carrier such as keyhole limpet hemocyanin (KLH) and used to immunize additional similar mice. Sera from these mice will contain anti-anti-Id antibodies that have the binding properties of the original MAb specific for a hFGF-20 epitope. The anti-Id MAbs thus have their own idiotypic epitopes, or "idiotopes" structurally similar to the epitope being evaluated, such as a hFGF-20 protein or glycoprotein.

The term "antibody" is also meant to include both intact molecules as well as fragments thereof, such as, for example, Fab and F(ab')$_2$, which are capable of binding antigen. Fab and F(ab')$_2$ fragments lack the Fc fragment of intact antibody, clear more rapidly from the circulation, and may have less non-specific tissue binding than an intact antibody (Wahl et al., J. Nucl. *Med.* 24:316-325 (1983)).

It will be appreciated that Fab and F(ab')a and other fragments of the antibodies useful in the present invention may be used for the detection and quantitation of a hFGF-20 protein or glycoprotein according to methods disclosed herein for intact antibody molecules. Such fragments are typeically produced by proteolytic cleavage, using enzymes

such as papain (to produce Fab fragments) or pepsin (to produce $F(ab')_2$ fragments).

A polypeptide used as an immunogen may be modified or administered in an adjuvant, by subcutaneous or intraperitoneal injection into, for example, a mouse or a rabbit. For the production of monoclonal antibodies, spleen cells from immunized animals are removed, fused with myeloma or other suitable known cells, and allowed to become monoclonal antibody producing hybridoma cells in the manner known to the skilled artisan. Hybridomas that secrete a desired antibody molecule can be screened by a variety of well-known methods, for example ELISA assay, Western blot analysis, or radioimmunoassay (Lutz, et al. Exp. Cell Res. 175:109-124 (1988); Monoclonal Antibodies: Principles & Applications, Ed. J. R. Birch & E. S. Lennox, Wiley-Liss (1995); Colligan, supra).

As is discussed herein, because of their amino acid sequence homologies to previously identified FGF polypeptides (i e., FGFs 1-9), as well as their tissue localizations, FGF-20 polypeptides are thought to play roles in regulating the development and function of the nervous system. Altered levels of FGF-20 nucleic acids and/or polypeptides, such as increased levels, may thus be associated with cell proliferative disorders, including, but not limited to, cell proliferative disorders of the nervous system. The terms "cell-proliferative disorder" or "proliferative disorder" are used herein to describe conditions that are characterized by abnormally excessive cell growth, including malignant, as well as non-malignant, cell growth. Conversely, conditions characterized by inadequate cell growth may be characterized by decreased expression of FGF-20s. These conditions can be diagnosed and monitored by detecting the levels of FGF-20s in patient samples. Accordingly, antibodies included in this invention

are useful in diagnostics, therapeutics or in diagnostic/therapeutic combinations.

Antibodies which recognize hFGF-20 epitopes and hFGF-9 polypeptide encoding nucleic acids can be used as probes in methods to detect the presence of an FGF-20 polypeptide (using an antibody) or nucleic acid (using a nucleic acid probe) in a sample, such as a biological fluid (e.g., cerebrospinal fluid (CSF), such as lumbar or ventricular CSF) or a tissue sample (e.g., CNS tissue, e.g., neural tissue or eye tissue). In these methods, an FGF-20 epitope recognizing antibody or nucleic acid probe is contacted with a sample from a patient suspected of having an FGF-20-associated disorder under conditions which favor specific binding of the antibody or nucleic acid probe to the molecule sought. The level of FGF-20 polypeptide or nucleic acid present in the suspect sample can be compared with the level in a control sample, e.g., an equivalent sample from an unaffected individual, to determine whether the patient has an FGF-20-associated cell proliferative disorder. FGF-20 polypeptides, or fragments thereof, can also be used as probes in diagnostic methods, for example, to detect the presence of FGF-20-specific antibodies in samples.

In one aspect, the present invention relates to a method for detecting the presence of or measuring the quantity of a hFGF-20 protein or glycoprotein in a cell, comprising:

(a) contacting said cell or an extract thereof with an antibody specific for an epitope of a hFGF-20 protein or glycoprotein; and

(b) detecting the binding of said antibody to said cell or extract thereof, or measuring the quantity of antibody bound, thereby determining the presence of or measuring the quantity of said hFGF-20 protein or glycoprotein.

FGF-20-specific antibodies, can also be used to screen

samples, e.g., expression libraries, for nucleic acids encoding novel FGF-20 polypeptides, or portions thereof. For example, an antibody that specifically binds to an FGF-20-specific peptide can be used in this method. Methods for carrying out such screening are well-known in the art (see, e.g., Ausubel, et al., supra).

Use of FGF-20-specific antibodies in diagnostic methods is described further, as follows. The antibodies of the invention can be used in vitro or in vivo for immunodiagnosis. The antibodies are suited for use in, for example, immunoassays in which they are in liquid phase or bound to a solid phase carrier (e.g., a glass, polystyrene, polypropylene, polyethylene, dextran, nylon, amylase, natural and modified cellulose, polyacrylamide, agarose, or magnetite carrier).

For some applications labeled antibodies are desirable. Procedures for labeling antibody molecules are widely known, including for example, the use of radioisotopes, affinity labels, such as biotin or avidin, enzymatic labels, for example horseradish peroxidase, and fluorescent labels, such as FITC or rhodamine (see, e.g., Colligan, supra). Labeled antibodies are useful for a variety of diagnostic applications. Examples of immunoassays in which the antibodies of the invention can be used include, e.g., competitive and non-competitive immunoassays, which are carried out using either direct or indirect formats. Examples of such immunoassays include radioimmunoassays (RIA) and sandwich assays (e.g., enzyme-linked immunosorbent assays (ELISAs)). Detection of antigens using the antibodies of the invention can be done using immunoassays that are run in either forward, reverse, or simultaneous modes, including immunohistochemical assays on physiological samples. Other immunoassay formats are well-known in the art, and can be used in the invention (see, e.g., Coligan, et al., supra).

In one embodiment the present invention relates to the use of labeled antibodies to detect the presence of an hFGF-20 polypeptide. Alternatively, the antibodies could be used in a screen to identify potential modulators of an hFGF-20 polypeptide. For example, in a competitive displacement assay, the antibody or compound to be tested is labeled by any suitable method. Competitive displacement of an antibody from an antibody-antigen complex by a test compound such that a test compound-antigen complex is formed provides a method for identifying compounds that bind hFGF-20.

In addition to the in vitro methods described above, FGF-20-specific monoclonal antibodies can be used in methods for in vivo detection of an antigen, such as an FGF-20 antigen. In these methods, a detectably labeled antibody is administered to a patient in a dose that is determined to be diagnostically effective by one skilled in the art. The term "diagnostically effective" is used herein to describe the amount of detectably labeled monoclonal antibody that is administered in a sufficient quantity to enable detection of the site having the antigen for which the monoclonal antibody is specific. As would be apparent to one skilled in the art, the concentration of detectably labeled monoclonal antibody that is administered should be sufficient so that the binding of the antibody to the cells containing the polypeptide is detectable, compared to background. Further, it is desirable that the detectably labeled monoclonal antibody is rapidly cleared from the circulatory system, to give the optimal target-to-background signal ratio. The dosage of detectably labeled monoclonal antibodies for in vivo diagnosis will vary, depending on such factors as the age and weight of the individual, as well as the extent of the disease. The dosages can also vary depending on factors such as whether multiple administrations are intended, antigenic burden, and other factors known to those of skill

in the art.

In addition to initial diagnosis, the FGF-20 polypeptides, nucleic acids, and FGF-20-specific antibodies described above can be used in in vitro or in vivo methods for monitoring the progress of a condition associated with FGF-20 expression. For example, they can be used in methods to monitor the course of amelioration of an FGF-20-associated disease, for example, after treatment has begun. In these methods, changes in the levels of an FGF-20-specific marker (e.g., an FGF-20 polypeptide, an FGF-20 nucleic acid, or an FGF-20-specific antibody) are detected, either in a sample from a patient or using the in vivo methods described above.

Identification of FGF-20-associating Polypeptides

Proteins that bind to hFGF-20 and/or a complex comprising hFGF-20 are potentially important neuronal regulatory proteins, receptors, or neuronal developmental proteins. These proteins are referred to herein as hFGF-20 associating proteins. Associating proteins may be isolated by various methods known in the art. Accordingly, the present invention also provides methods for identifying polypeptides which bind to a hFGF-20 polypeptide.

A preferred method of isolating associating proteins is by contacting an hFGF-20 polypeptide to an antibody that binds the hFGF-20 polypeptide, and isolating resultant immune complexes. These immune complexes may contain associating proteins bound to the hFGF-20 polypeptide. The associating proteins may be identified and isolated by denaturing the immune complexes with a denaturing agent and, preferably, a reducing agent. The denatured, and preferably reduced, proteins can be separated on a polyacrylamide gel. Putative hFGF-9 polypeptide associating proteins are then identified on the polyacrylamide gel by one or more of

various well-known methods (e.g., Coomassie staining, Western blotting, silver staining, etc.) and isolated by resection of a portion of the polyacrylamide gel containing the relevant identified polypeptide and elution of the polypeptide from the gel portion.

A putative FGF-20 associating protein may be identified as an associating protein by demonstration that the protein binds to hFGF-20 and/or a complex comprising hFGF-20. Such binding may be shown in vitro by various means, including, but not limited to, binding assays employing a putative associating protein that has been renatured subsequent to isolation by a polyacrylamide gel electrophoresis method. Alternatively, binding assays employing recombinant or chemically synthesized putative associating protein may be used. For example, a putative associating protein may be isolated and all or part of its amino acid sequence determined by chemical sequencing, such as Edman degradation. The amino acid sequence information may be used to chemically synthesize the putative associating protein or to produce a recombinant putative hFGF-20 associating protein.

hFGF-20 associating proteins may also be identified by cross-linking *in vivo* with bi-functional cross-linking reagents (e.g., dimethylsuberimidate, glutaraldehyde, etc.) and subsequent isolation of cross-linked products that include a hFGF-20 polypeptide. (For a general discussion of cross-linking, see Kunkel, et al, Mol. Cell. Biochem, 34:3 (1981), which is incorporated herein by reference). Preferably, the bi-functional cross-linking reagent will produce cross-links that may be reversed under specific conditions after isolation of the cross-linked complex so as to facilitate isolation of the associating protein from the Lyar polypeptide. Isolation of cross-linked complexes that include a hFGF-20 polypeptide is preferably accomplished by

binding an antibody that binds an hFGF-20 polypeptide with an affinity of at least 1 x 10^7 M^{-1} to a population of cross-linked complexes and recovering only those complexes that bind to the antibody with an affinity of at least 1 x 10^7 M^{-1}. Polypeptides that are cross-linked to a hFGF-20 polypeptide are identified as hFGF-20 associating proteins.

Also, an expression library, such as a λgt11 cDNA expression library (Dunn, et al., J. Biol. Chem. 264: 13057 (1989)), can be screened with a labeled hFGF-20 polypeptide to identify cDNAs encoding polypeptides which specifically bind to the hFGF-20 polypeptide. For these procedures, cDNA expression libraries usually comprise mammalian cDNA populations, typically human, mouse, or rat, and may represent cDNA produced from RNA of one cell type, tissue, or organ and one or more developmental stage. Specific binding for screening cDNA expression libraries is usually provided by including one or more blocking agent (e.g., albumin, nonfat dry milk solids, etc.) prior to and/or concomitant with contacting the labeled hFGF-20 polypeptide (and/or labeled anti-hFGF-20 antibody).

Another approach to identifying polypeptide sequences which bind to a predetermined polypeptide sequence (i.e., hFGF-20) has been to use a variation on the so-called "two-hybrid" system. Two-hybrid methods generally rely upon a positive association between two fusion proteins thereby reconstituting a functional transcriptional activator which then induces transcription of a reporter gene operably linked to an appropriate transcriptional activator binding site. Transcriptional activators are proteins that positively regulate the expression of specific genes. They can be functionally dissected into two structural domains: one region that binds to specific DNA sequences and thereby confers specificity, and another region termed the activation domain that binds to protein components of the

basal gene expression machinery (Ma and Ptashne, Cell, 55: 443 (1988)). These two domains need to be physically connected in order to function as a transcriptional activator. Two-hybrid systems exploit this requirement by hooking up an isolated DNA binding domain to one protein (protein X), while hooking up the isolated activation domain to another protein (protein Y). When X and Y interact to a significant extent, the DNA binding and activation domains will now be connected and the transcriptional activator function reconstituted. The host strain is engineered so that the reconstituted transcriptional activator drives the expression of a specific reporter gene, which provides the read-out for the protein-protein interaction (Field and Song, (1989); Chein et al., (1991)). Transcription of the reporter gene produces a positive readout, typically manifested either (1) as an enzyme activity (e.g., β-galactosidase) that can be identified by a calorimetric enzyme assay or (2) as enhanced cell growth on a defined medium (e.g., HIS3). A positive readout condition is generally identified as, but not limited to, one or more of the following detectable conditions: (1) an increased transcription rate of a predetermined reporter gene, (2) an increased concentration or abundance of a polypeptide product encoded by a predetermined reporter gene, typically an enzyme which can be readily assayed in vivo, and/or (3) a selectable or otherwise identifiable phenotypic change in a organism harboring the two-hybrid system. Generally, a selectable or otherwise identifiable phenotypic change that characterizes a positive readout condition confers upon the organism (e.g., yeast, bacteria, mammalian cell) either: a selective growth advantage on a defined medium, a mating phenotype, a characteristic morphology or developmental stage, drug resistance, or a detectable enzymatic activity (e.g., β-galactosidase, luciferase, alkaline phosphatase).

One advantage of a two-hybrid system for monitoring protein-protein interactions is their sensitivity in detection of physically weak, but physiologically important, protein-protein interactions. As such it offers a significant advantage over other methods for detecting protein-protein interactions (e.g., ELISA assay).

Typically, the two-hybrid method is used to identify novel polypeptide sequences which interact with a known protein (Silver S.C. and Hunt S.W., Mol. Biol. Rep., 17:155 (1993); Durfee et al., Genes Devel., 7;555 (1993); Yang et al., Science, 257:680 (1992); Luban et al., Cell 73:1067 (1993); Hardy et al., Genes Devel., 6:801 (1992); Bartel et al., Biotechniques, 14:920 (1993); and VojTek et al., Cell 74:205 (1993)). However, two hybrid systems have also been used to identify interacting structural domains of known proteins (Bardwell et al., Med. Microbio., 8:1177 (1993); Chakraborty et al., J. Biol. Chem., 267:17498 (1992); Staudinger et al., J. Biol. Chem., 268:4608 (1993); and Milne, G.T. and Weaver, D.T. Genes Devel. 7:1755 (1993)) or domains responsible for oligomerization of a single protein (Iwabuchi et al., Oncogene, 8:1693 (1993); Bogerd et al., J. Virol., 67:5030 (1993)).

A preferred two-hybrid system identifies protein-protein interactions in vivo through reconstitution of a transcriptional activator, the yeast Gal4 transcription protein (Fields and Song, Nature, (1989)). The yeast Gal4 protein consists of separable domains responsible for DNA-binding and transcriptional activation. Polynucleotides encoding two hybrid proteins, one consisting of the yeast Gal4 DNA-binding domain fused to a polypeptide sequence of a known protein and the other consisting of the Gal4 activation domain fused to a polypeptide sequence of a second protein, are constructed and introduced into a yeast host cell. Intermolecular binding between the two fusion

proteins reconstitutes the Ga14 DNA-binding domain with the Ga14 activation domain, which leads to the transcriptional activation of a reporter gene (e.g., lacZ, HIS3) which is operably linked to a Ga14 binding site.

Alternatively, an E. coli/BCCP interactive screening system or other variations on the two-hybrid system known in the art can be used to identify interacting protein sequences. (See, e.g., Germino et al., Proc. Natl. Acad. Sci. (USA), 90:933 (1993); Guarente, L., Proc. Natl. Acad. Sci. (USA), 90:1639 (1993); Frederickson, R.M., Current Opinion in Biotechnology, 9(1):90-6 (1998); Vidal, M. and Legrain P. Nucleic Acids Research, 27(4):919-29 (1999); Drees, B.L. Current Opinion in Chemical Biology, 3(1):64-70 (1999); Sorimachi H., et al., Protein, Nucleic Acid, Enzyme, 42(14 Suppl):2433-40 (1997), each entirely incorporated herein by reference).

For the above mentioned procedures, expression libraries usually comprise mammalian cDNA populations, typically human, mouse, simian, or rat, and may represent cDNA produced from RNA of one or more cell type, tissue, or organ and one or more developmental stage. Specific binding for screening cDNA expression libraries is usually provided by including one or more blocking agent (e.g., albumin, nonfat dry milk solids, etc.) prior to and/or concomitant with contacting the labeled hFGF-20 polypeptide (and/or labeled anti-hFGF-20 antibody).

Also included in the present invention are the multitude of screening assays which one skilled in the art can develop to identify compounds which inhibit or induce binding of hFGF-20 to hFGF-20 associating proteins (under suitable binding conditions) based on the disclosures provided herein including, but not limited to, any one of the aforementioned protein-protein interaction assays comprising hFGF-20 polynucleotides, polypeptides, and/or

antibodies.

Transgenics and Chimeric Non-Human Mammals

Another embodiment of the present invention provides transgenic non-human mammals carrying a recombinant hFGF-20 gene construct in its somatic and germ cells. The recombinant gene construct may be composed of regulatory DNA sequences that belong to the native hFGF-20 gene or those which are derived from an alternative source. These regulatory sequences are functionally linked to the hFGF-20 coding region, resulting in the constitutive and/or regulatable expression of hFGF-20 in the body of the transgenic non-human mammal. The most important of such regulatory sequences is the promoter. Promoters are defined in this context as any and all DNA elements necessary for the functional expression of a gene. Promoters drive the expression of structural genes and may be modulated by inducers and repressors. Numerous promoters have been described in the literature and are easily within the grasp of the ordinarily skilled artisan. Viral promoters, such as the SV40 early promoter, are consistent with the invention though mammalian promoters are preferred. The promoter is chosen such that the level of expression is sufficient to promote physiological consequences in the transgenic non-human mammal, or ancestor of said mammal. Preferably, the genome of the transgenic mammal contains at least 30 copies of a transgene. More preferably, the genome of the transgenic mammal contains at least 50 copies, and may contain 100-200 or more copies of the transgene. Generally, said nucleic acid is introduced into said mammal at an embryonic stage, preferably the 1-1000 cell or oocyte stage, and, most preferably not later than about the 64-cell stage. Most preferably the transgenic mammal is homozygous for the transgene.

The techniques described in Leder, U.S. Patent No. 4,736,866 (hereby entirely incorporated by reference) for producing transgenic non-human mammals may be used for the production of a transgenic non-human mammal of the present invention. The various techniques described in U.S. patent Nos. 5,454,807, 5,073,490, 5,347,075, 4870,009, and 4,736,866, the entire contents of which are hereby incorporated by reference, may also be used. Such methods are also described in Methods in Molecular Biology, Vol. 18, 1993, Transgenesis Techniques, Principles and Protocols, (Murphy, D., and Carter, D.A.) as well as in U.S. Patents #5,174,986, #5,175,383, #5,175,384, and #5,175,385, all of which are herein incorporated by reference.

Also intended to be within the scope of the present invention are chimeric non-human mammals in which fewer than all of the somatic and germ cells contain a DNA construct comprising a nucleic acid encoding a hFGF-20 polypeptide of the present invention. Contemplated chimeric non-human mammals include animals produced when fewer than all of the cells of the morula are transfected in the process of producing the transgenic animal.

Transgenic and chimeric non-human mammals having human cells or tissue engrafted therein are also encompassed by the present invention. Methods for providing chimeric non-human mammals are provided, e.g., in U.S. Serial Nos. 07/508,225, 07/518,748, 07/529,217, 07/562,746, 07/596,518, 07/574,748, 07/575,962, 07/207,273, 07/241,590 and 07/137,173, which are entirely incorporated herein by reference, for their description of how to engraft human cells or tissue into non-human mammals.

Alternatively, genetic constructs comprising at least one of the hFGF-20 nucleic acid sequences as defined herein may be used to create transgenic "knockouts" of the hFGF-20 gene. Accordingly, the present invention also provides a

transgenic animal which has been engineered by homologous recombination to be deficient in the expression of the endogenous FGF-20 gene. Further, the invention provides a method of producing an heterozygous or homozygous transgenic animal deficient in or lacking functional FGF-20 proteins, respectfully, said method comprising:

a) obtaining a DNA construct comprising a disrupted hFGF-20 gene, wherein said disruption is by the insertion of an heterologous marker sequence;

b) introducing said DNA construct into an ES cell of said animal such that the endogenous FGF-20 gene is disrupted by homologous recombination;

c) selecting ES cells comprising said disrupted allele;

d) incorporating the ES cells of step c) into a mouse embryo;

e) transferring said embryo into a pseudopregnant animal of the said species;

f) developing said embryo into a viable offspring;

g) screening offspring to identify heterozygous animal comprising said disrupted FGF-20 gene; and

h) if desired, breeding said heterozygous animal to produce homozygous transgenic animals of said species, wherein the said homozygous animal does not express functional FGF-20 proteins.

Transgenic and chimeric non-human mammals of the present invention may be used for analyzing the consequences of over-expression of at least one hFGF-20 polypeptide *in vivo*. Such animals are also useful for testing the effectiveness of therapeutic and/or diagnostic agents, either associated or unassociated with delivery vectors or vehicles, which preferentially bind to an hFGF-20 polypeptide of the present invention or act to indirectly modulate hFGF-20 activity.

hFGF-20 transgenic non-human mammals are useful as an animal models in both basic research and drug development endeavors. Transgenic animals carrying at least one hFGF-20 polypeptide or nucleic acid can be used to test compounds or other treatment modalities which may prevent, suppress, or cure a pathology or disease associated with at least one of the above mentioned hFGF-20 activities. Such transgenic animals can also serve as a model for the testing of diagnostic methods for those same diseases. Furthermore, tissues derived from hFGF-20 transgenic non-human mammals are useful as a source of cells for cell culture in efforts to develop *in vitro* bioassays to identify compounds that modulate hFGF-20 activity or hFGF-20 dependent signaling. Accordingly, another aspect of the present invention contemplates a method of identifying compounds efficacious in the treatment of at least one previously described disease or pathology associated with FGF-20 activity. A non-limiting example of such a method comprises:

a) generating an hFGF-20 transgenic non-human animal which is, as compared to a wild-type animal, pathologically distinct in some detectable or measurable manner from wild-type version of said non-human mammal;

b) exposing said transgenic animal to a compound, and;

c) determining the progression of the pathology in the treated transgenic animal, wherein an arrest, delay, or reversal in disease progression in transgenic animal treated with said compound as compared to the progression of the pathology in an untreated control animals is indicative that the compound is useful for the treatment of said pathology

Another embodiment of the present invention provides a method of identifying compounds capable of inhibiting hFGF-20 activity *in vivo* and/or *in vitro* wherein said method comprises:

a) administering an experimental compound to an hFGF-20 transgenic non-human animal, or tissues derived therefrom, exhibiting one or more physiological or pathological conditions attributable to the overexpression of an hFGF-20 transgene; and

b) observing or assaying said animal and/or animal tissues to detect changes in said physiological or pathological condition or conditions.

Another embodiment of the invention provides a method for identifying compounds capable of overcoming deficiencies in hFGF-20 activity *in vivo* or *in vitro* wherein said method comprises:

a) administering an experimental compound to an hFGF-20 transgenic non-human animal, or tissues derived therefrom, exhibiting one or more physiological or pathological conditions attributable to the disruption of the endogenous FGF-20 gene; and

b) observing or assaying said animal and/or animal tissues to detect changes in said physiological or pathological condition or conditions.

Various means for determining a compound's ability to modulate hFGF-20 in the body of the transgenic animal are consistent with the invention. Observing the reversal of a pathological condition in the transgenic animal after administering a compound is one such means. Another more preferred means is to assay for markers of hFGF-20 activity in the blood of a transgenic animal before and after administering an experimental compound to the animal. The level of skill of an artisan in the relevant arts readily provides the practitioner with numerous methods for assaying physiological changes related to therapeutic modulation of hFGF-20 activity.

In all previously described *in vitro* and *in vivo* assays, the compound may be administered when applicable, either superficially, orally, parenterally (e.g. by intravenous infusion or injection) or a combination of injection and infusion (iv), intramuscularly (im), or subcutaneously (sc). A preferred route of compound administration to an animal is iv, while oral administration is most preferred.

Nucleic Acid Diagnosis and Diagnostic Kits

According to the diagnostic and prognostic methods of the present invention, an alteration of the wild-type FGF-20 locus is detected. In addition, the method can be performed by detecting the wild-type FGF-20 locus and confirming the lack of a predisposition to a particular neurological disorder or neoplasia.

Genetic mutations can manifest themselves in several forms in the coding and/or non-coding regions. The finding of FGF-20 mutations thus provides both diagnostic and prognostic information. An FGF-20 allele which is not deleted (e.g., that found on the sister chromosome to a chromosome carrying an FGF-20 deletion) can be screened for other mutations, such as insertions, small deletions, and point mutations. It is believed that many mutations found in cancerous tissues will be those leading to decreased or increased expression of the hFGF-20 gene product. However, mutations leading to non-functional gene products would also lead to a cancerous state. Point mutational events may occur in regulatory regions, such as in the promoter of the gene, leading to loss or diminution of expression of the mRNA. Point mutations may also abolish proper RNA processing, leading to loss of expression of the FGF-20 gene product, or a decrease in mRNA stability or translation efficiency. In general point mutations represent the most

difficult type of mutation to screen for and detect because they represent the smallest degree of molecular change. A number of genetic defects can be linked to a specific single point mutation within a gene, e.g. sickle cell anemia while many others are caused by a wide spectrum of different mutations throughout the gene. Many procedures have been developed for scanning genes in order to detect mutations, which are applicable to both exons and introns.

Useful diagnostic techniques include, but are not limited to fluorescent in situ hybridization (FISH), direct DNA sequencing, PFGE analysis, Southern blot analysis, single stranded conformation analysis (SSCA), RNase protection assay, allele-specific oligonucleotide (ASO), dot blot analysis and PCR-SSCP, as discussed in detail further below.

A rapid preliminary analysis to detect polymorphisms in DNA sequences can be performed by looking at a series of Southern blots of DNA cut with one or more restriction enzymes, preferably a large number of restriction enzymes. Each blot contains a series of normal individuals and a series of cancer cases, tumors, or both. Southern blots displaying hybridizing fragments (differing in length from control DNA when probed with sequences near or including the FGF-20 locus) indicate a possible mutation. If restriction enzymes which produce very large restriction fragments are used, then pulsed field gel electrophoresis ("PFGE") is employed.

Detection of point mutations may be accomplished by molecular cloning of the FGF-20 allele(s) and sequencing that allele(s) using techniques well-known in the art. Alternatively, the gene sequences can be amplified, using known techniques, directly from a genomic DNA preparation from the tumor tissue. The DNA sequence of the amplified sequences can then be determined. Sequencing reactions can

be performed to screen the full genetic target base by base. This process, which can pinpoint the exact location and nature of mutation, requires labeling DNA, use of polyacrylamide gels, and a multiplicity of reactions to assess all bases over the length of a gene, all of which are slow and labor intensive procedures. (J. Bergh et al. "Complete Sequencing of the p 53 Gene Provides Prognostic Information in Breast Cancer Patients, Particularly in Relation to Adjuvant Systemic Therapy and Radiotherapy," Nature Medicine 1, 1029 (1995)). Insertions and deletions of genes can also be detected by cloning, sequencing and amplification. In addition, restriction fragment length polymorphism (RFLP) probes for the gene or surrounding marker genes can be used to score alteration of an allele or an insertion in a polymorphic fragment. Such a method is particularly useful for screening relatives of an affected individual for the presence of the FGF-20 mutation found in that individual. Other techniques for detecting insertions and deletions as known in the art can be used.

There are six well-known methods for a more complete, yet still indirect, test for confirming the presence of a susceptibility allele: 1) single stranded conformation analysis ("SSCA") (Orita et al., 1989); 2) denaturing gradient gel electrophoresis ("DGGE") (Wartell et al., 1990; Sheffield et al., 1989); 3) RNase protection assays (Finkelstein et al., 1990; Kinszler et al., 1991); 4) allele-specific oligonucleotides ("ASOs") (Conner et al., 1983); 5) the use of proteins which recognize nucleotide mismatches, such as the E. coli mutS protein (Modrich, 1991); and, 6) allele-specific PCR (Rano & Kidd, 1989). For allele-specific PCR, primers are used which hybridize at their 3' ends to a particular FGF-20 mutation. If the particular FGF-20 mutation is not present, an amplification product is not observed. Amplification Refractory Mutation

System (ARMS) can also be used, as disclosed in European Patent Application Publication No. 0332435 and in Newton et al., 1989.

The single strand conformational (SSCP) polymorphism assay takes advantage of structural variation within DNA that results from mutation. The method involves folding the single-stranded form of a given nucleic acid sequence into a thermodynamically directed secondary and tertiary structure. In most cases, mutated sequences form different structures than the wild type sequence, thus permitting separation of mutated and wild type sequences by gel electrophoresis. (M. Orita, et al., "Detection of Polymorphisms of Human DNA by Gel Electrophoresis as Single-Stranded Conformation Polymorphisms," Proc. Natl. Acad. Sci. USA 86, 2766 (1989). Like SSCP, DGGE assays also differentiate based on structural variation. The different thermodynamic stability of structures formed by the mutant sequence, as opposed to wild type, lead to differences in the temperature and/or pH at which the molecule will denature. DGGE mutation identification and localization properties are similar to those for SSCP though sensitivity is higher for DGGE because not all mutations cause the structural changes that the SSCP method depends upon for detection. (E. S. Abrams, S. E. Murdaugh & L. S. Lerman, "Comprehensive Detection of Single Base Changes in Human Genomic DNA Using Denaturing Gradient Gel Electrophoresis and a GC Clamp," Genomics 7, 463 (1990)).

The RNase protection involves the use of a labeled riboprobe which is complementary to the human wild-type FGF-20 gene coding sequence. The riboprobe and either mRNA or DNA isolated from the tumor tissue are annealed (hybridized) together and subsequently digested with the enzyme RNase A which is able to detect some mismatches in a duplex RNA structure. If a mismatch is detected by RNase A, it cleaves

at the site of the mismatch. Thus, when the annealed RNA preparation is separated on an electrophoretic gel matrix, if a mismatch has been detected and cleaved by RNase A, an RNA product will be seen which is smaller than the full length duplex RNA for the riboprobe and the mRNA or DNA. The riboprobe need not be the full length of the FGF-20 mRNA or gene but can be a segment of either. If the riboprobe comprises only a segment of the FGF-20 mRNA or gene, it will be desirable to use a number of these probes to screen the whole mRNA sequence for mismatches.

In similar fashion, DNA probes can be used to detect mismatches, through enzymatic or chemical cleavage. See, e.g., Cotton et al., 1988; Shenk et al., 1975; Novack et al., 1986. Alternatively, mismatches can be detected by shifts in the electrophoretic mobility of mismatched duplexes relative to matched duplexes. See, e.g., Cariello, 1988. With either riboprobes or DNA probes, the cellular mRNA or DNA which might contain a mutation can be amplified using PCR (see below) before hybridization. Changes in DNA of the FGF-20 gene can also be detected using Southern hybridization, especially if the changes are gross rearrangements, such as deletions and insertions.

In an allele-specific oligonucleotide assay, an oligonucleotide is designed which detects a specific sequence, and the assay is performed by detecting the presence or absence of a hybridization signal. DNA sequences of the FGF-20 gene which have been amplified by use of PCR may also be screened using allele-specific probes. These probes are nucleic acid oligomers, each of which contains a region of the FGF-20 gene sequence harboring a known mutation. For example, one oligomer may be about 30 nucleotides in length, corresponding to a portion of the FGF-20 gene sequence. By use of a battery of such allele-specific probes, PCR amplification products can be

screened to identify the presence of a previously identified mutation in the FGF-20 gene. Hybridization of allele-specific probes with amplified FGF-20 sequences can be performed, for example, on a nylon filter. Hybridization to a particular probe under stringent hybridization conditions indicates the presence of the same mutation in the tumor tissue as in the allele-specific probe.

In the mutS assay, the protein binds only to sequences that contain a nucleotide mismatch in a heteroduplex between mutant and wild-type sequences. Mismatches, according to the present invention, are hybridized nucleic acid duplexes in which the two strands are not 100% complementary. Lack of total homology may be due to deletions, insertions, inversions or substitutions. Mismatch detection can be used to detect point mutations in the gene or its mRNA product. While these techniques are less sensitive than sequencing, they are simpler to perform on a large number of tumor samples.

The most definitive test for mutations in a candidate locus is to directly compare genomic FGF-20 sequences from cancer patients with those from a control population. Alternatively, one could sequence messenger RNA after amplification, e.g., by PCR, thereby eliminating the necessity of determining the exon structure of the candidate gene. Mutations from cancer patients falling outside the coding region of FGF-20 can be detected by examining the non-coding regions, such as introns and regulatory sequences near or within the FGF-20 gene. An early indication that mutations in noncoding regions are important may come from Northern blot experiments that reveal messenger RNA molecules of abnormal size or abundance in cancer patients as compared to control individuals. Alteration of FGF-20 mRNA expression can be detected by any techniques known in the art. These include Northern blot analysis, PCR

amplification and RNase protection. Diminished mRNA expression indicates an alteration of the wild-type FGF-20 gene. Mutant FGF-20 genes or gene products can also be detected in other human body samples, such as serum, stool, urine and sputum. The same techniques discussed above for detection of mutant FGF-20 genes or gene products in tissues can be applied to other body samples. Cancer cells are sloughed off from tumors and appear in such body samples. In addition, the FGF-20 gene product itself may be secreted into the extracellular space and found in these body samples even in the absence of cancer cells. By screening such body samples, a simple early diagnosis can be achieved for many types of cancers. In addition, the progress of chemotherapy or radiotherapy can be monitored more easily by testing such body samples for mutant FGF-20 genes or gene products.

The methods of diagnosis of the present invention are applicable to any tumor in which FGF-20 has a role in tumorigenesis. Deletions of chromosome arm 8p or somatic mutations within the FGF-20 region have been observed in tumors. The diagnostic method of the present invention is useful for clinicians, so they can decide upon an appropriate course of treatment. The pairs of single-stranded DNA primers can be annealed to sequences within or surrounding the FGF-20 gene on chromosome 8p in order to prime amplifying DNA synthesis of the FGF-20 gene itself. A complete set of these primers allows synthesis of all of the nucleotides of the FGF-20 gene coding sequences, i.e., the exons. The set of primers preferably allows synthesis of both intron and exon sequences. Allele-specific primers can also be used. Such primers anneal only to particular FGF-20 mutant alleles, and thus will only amplify a product in the presence of the mutant allele as a template.

Several approaches to screening for mutations involve the probing of a target nucleic acid by an array of

oligonucleotides that can differentiate between normal wild type nucleic acids and mutant nucleic acids. These arrays involve the performance of hundreds or thousands of hybridization reactions in parallel with different site-directed oligonucleotides and requires sophisticated and costly probe arrays. Hybridization arrays can identify the location and type of mutation in many, but not all cases. For example, semihomologous sequential insertions or targets with repeating sequences and/or repeating sequential motifs cannot be analyzed by hybridization. (A. C. Pease et al., "Light-Generated Oligonucleotide Arrays for Rapid DNA Sequence Analysis," Proc. Natl. Acad. Sci. USA 91, 5022 (1994)). Tailor-made processes that focus on the use of mass spectrometry are suitable for genetic screening tests. Mass spectrometry requires minute samples, provides extremely detailed information about the molecules being analyzed including high mass accuracy, and is easily automated. K. Tanaka et al., "Protein and Polymer Analyses up to m/z 100,000 by Laser Ionization Time-of-flight Mass Spectrometry," Rapid Commun. Mass Spectrom. 2, 151-153 (1988); B. Spengler et al., "Laser Mass Analysis in Biology," Ber. Bunsenges. Phys. Chem. 93, 396-402 (1989); J. B. Fenn et al., Science 246, 64-71 (1989); Wu, et al., Rapid Comm'ns in Mass Spectrometry, 7:142-146 (1993); U.S. Patent Nos. 5,869,242, 5, 885, 775, and 6,051,378).

For mutations localized within a given gene, such as the cystic fibrosis DELTA.F508 deletion, it is also possible to perform a single PCR or ligase chain reaction (LCR) assay or simple hybridization assays tailored to these specific sites. PCR and LCR results are presently determined by the use of labeled molecules, where radioactive emissions, fluorescence, chemiluminescence or color changes are detected directly. (P. Fang et al., "Simultaneous Analysis of Mutant and Normal Alleles for Multiple Cystic Fibrosis

Mutations by the Ligase Chain Reaction," Human Mutation 6, 144 (1995)).

In order to detect the presence of a FGF-20 allele predisposing an individual to at least one of the neurological disorders or cancers described herein, a biological sample such as blood is prepared and analyzed for the presence or absence of susceptibility alleles of FGF-20. In order to detect the presence of neoplasia, the progression toward malignancy of a precursor lesion, or as a prognostic indicator, a biological sample of the lesion is prepared and analyzed for the presence or absence of neoplastic alleles of FGF-20.

Both PCR and non-PCR based screening strategies can detect target sequences with a high level of sensitivity. Generally, the most popular method used today is target amplification. Here, the target nucleic acid sequence is amplified with polymerases. Once amplified, the resulting nucleic acid can be sequenced or used as a substrate for DNA probes. When the probes are used to detect the presence of the target sequences (for example, in screening for cancer susceptibility), the biological sample to be analyzed, such as blood or serum, may be treated, if desired, to extract the nucleic acids. The sample nucleic acid may be prepared in various ways to facilitate detection of the target sequence; e.g. denaturation, restriction digestion, electrophoresis or dot blotting. The targeted region of the analyte nucleic acid usually must be at least partially single-stranded to form hybrids with the targeting sequence of the probe. If the sequence is naturally single-stranded, denaturation will not be required. However, if the sequence is double-stranded, the sequence will probably need to be denatured. Denaturation can be carried out by various techniques known in the art. Analyte nucleic acid and probe are incubated under conditions which promote stable hybrid

formation of the target sequence in the probe with the putative targeted sequence in the analyte. The region of the probes which is used to bind to the analyte can be made completely complementary to the targeted region of human chromosome 8p. Therefore, high stringency conditions are desirable in order to prevent false positives. However, conditions of high stringency are used only if the probes are complementary to regions of the chromosome which are unique in the genome. The stringency of hybridization is determined by a number of factors during hybridization and during the washing procedure, including temperature, ionic strength, base composition, probe length, and concentration of formamide. These factors are outlined in, for example, Maniatis et al., 1982 and Sambrook et al., 1989. Under certain circumstances, the formation of higher order hybrids, such as triplexes, quadraplexes, etc., may be desired to provide the means of detecting target sequences.

Detection, if any, of the resulting hybrid is usually accomplished by the use of labeled probes. Alternatively, the probe may be unlabeled, but may be detectable by specific binding with a ligand which is labeled, either directly or indirectly. Suitable labels, and methods for labeling probes and ligands are known in the art, and include, for example, radioactive labels which may be incorporated by known methods (e.g., nick translation, random priming or kinasing), biotin, fluorescent groups, chemiluminescent groups (e.g., dioxetanes, particularly triggered dioxetanes), enzymes, antibodies and the like. Variations of this basic scheme are known in the art, and include those variations that facilitate separation of the hybrids to be detected from extraneous materials and/or that amplify the signal from the labeled moiety. A number of these variations are reviewed in, e.g., Matthews & Kricka, 1988; Landegren et al., 1988; Mittlin, 1989; U.S. Pat. No.

4,868,105, and in EPO Publication No. 225,807.

As noted above, non-PCR based screening assays are also contemplated in this invention. An exemplary non-PCR based procedure hybridizes a nucleic acid probe (or an analog such as a methyl phosphonate backbone replacing the normal phosphodiester), to the low level DNA target. This probe may have an enzyme covalently linked to the probe, such that the covalent linkage does not interfere with the specificity of the hybridization. This enzyme-probe-conjugate-target nucleic acid complex can then be isolated away from the free probe enzyme conjugate and a substrate is added for enzyme detection. Enzymatic activity is observed as a change in color development or luminescent output. For an example relating to the preparation of oligodeoxynucleotide-alkaline phosphatase conjugates and their use as hybridization probes see Jablonski et al., 1986.

Two-step label amplification methodologies are also known in the art. These assays work on the principle that a small ligand (such as digoxigenin, biotin, or the like) is attached to a nucleic acid probe capable of specifically binding FGF-20. In one example, the small ligand attached to the nucleic acid probe is specifically recognized by an antibody-enzyme conjugate. In one embodiment of this example, digoxigenin is attached to the nucleic acid probe. Hybridization is detected by an antibody-alkaline phosphatase conjugate which turns over a chemiluminescent substrate. In a second example, the small ligand is recognized by a second ligand-enzyme conjugate that is capable of specifically complexing to the first ligand. A well-known embodiment of this example is the biotin-avidin type of interactions.

It is also contemplated within the scope of this invention that the nucleic acid probe assays of this invention will employ a cocktail of nucleic acid probes

capable of detecting FGF-20 genes. Thus, in one example, to detect the presence of FGF-20 in a cell sample, more than one probe complementary to FGF-20 is employed and in particular the number of different probes is alternatively 2, 3, or 5 different nucleic acid probe sequences. In another example, to detect the presence of mutations in the FGF-20 gene sequence in a patient, more than one probe complementary to FGF-20 is employed where the cocktail includes probes capable of binding to the allele-specific mutations identified in populations of patients with alterations in FGF-20. In this embodiment, any number of probes can be used, and will preferably use multiple probes corresponding to the major gene mutations identified as predisposing an individual to at least one of the neurological disorders or cancers described herein.

Peptide Diagnosis and Diagnostic Kits

Alteration of wild-type FGF-20 genes can also be detected by screening for alteration of wild-type FGF-20 protein. For example, monoclonal antibodies immunoreactive with FGF-20 can be used to screen a tissue. Lack of cognate antigen would indicate an FGF-20 mutation. Antibodies specific for products of mutant alleles could also be used to detect mutant FGF-20 gene product. Such immunological assays can be done in any convenient formats known in the art. These include Western blots, immunohistochemical assays and ELISA assays. Any means for detecting an altered FGF-20 protein can be used to detect alteration of wild-type FGF-20 genes. Functional assays, such as protein binding determinations, can also be used.

The neoplastic condition of lesions can also be detected on the basis of the alteration of wild-type FGF-20 polypeptide. Such alterations can be determined by sequence analysis in accordance with conventional techniques. More

preferably, antibodies (polyclonal or monoclonal) are used to detect differences in, or the absence of FGF-20 peptides. In a preferred embodiment of the invention, antibodies will immunoprecipitate FGF-20 proteins from solution as well as react with FGF-20 protein on Western or immunoblots of polyacrylamide gels. In another preferred embodiment, antibodies will detect FGF-20 proteins in paraffin or frozen tissue sections, using immunocytochemical techniques. Techniques for raising and purifying antibodies are well-known in the art, and any such techniques may be chosen to achieve the preparation of the invention. Preferred embodiments relating to methods for detecting FGF-20 or its mutations include enzyme linked immunosorbent assays (ELISA), radioimmunoassays (RIA), immunoradiometric assays (IRMA) and immunoenzymatic assays (IEMA), including sandwich assays using monoclonal and/or polyclonal antibodies. Exemplary sandwich assays are described by David et al., in U.S. Pat. Nos. 4,376,110 and 4,486,530, hereby incorporated by reference.

Biological and Therapeutic Uses

The invention also provides methods for treating conditions associated with pathological expression of FGF-20 polypeptides, including cell proliferative disorders (e.g., including, but not limited to, those of the central or peripheral nervous systems, conditions affecting neural tissue, testes, heart tissue, cells of the eye, wounds or wound healing, as well as tumorigenesis and fibrotic diseases such as, but not limited to, asthma and renal failure). Treatment of an FGF-20-associated cell proliferative disorder can be carried out, for example, by modulating FGF-20 gene expression or FGF-20 activity in a cell. The term "modulate" includes, for example, suppressing expression of an FGF-20 when it is over-

expressed, and augmenting expression of an FGF-20 when it is under-expressed. In cases where a disorder is associated with over-expression of an FGF-20, nucleic acids that interfere with FGF-20 expression, at transcriptional or translational levels, can be used to treat the disorder. This approach employs, for example, antisense nucleic acids (i.e., nucleic acids that are complementary to, or capable of hybridizing with, a target nucleic acid, e.g., a nucleic acid encoding an FGF-20 polypeptide), ribozymes, or triplex agents. The antisense and triplex approaches function by masking the nucleic acid, while the ribozyme strategy functions by cleaving the nucleic acid. In addition, antibodies that bind to FGF-20 polypeptides can be used in methods to block the activity of an FGF-20.

The use of antisense methods to inhibit the in vitro translation of genes is well-known in the art (see, e.g., Marcus-Sakura, Anal. Biochem., 172:289, 1988). Antisense nucleic acids are nucleic acid molecules (e.g., molecules containing DNA nucleotides, RNA nucleotides, or modifications (e.g., modification that increase the stability of the molecule, such as 2'-O-alkyl (e.g., methyl) substituted nucleotides) or combinations thereof) that are complementary to, or that hybridize to, at least a portion of a specific nucleic acid molecule, such as an RNA molecule (e.g., an mRNA molecule) (see, e.g., Weintraub, Scientific American, 262:40, 1990). The antisense nucleic acids hybridize to corresponding nucleic acids, such as mRNAs, to form a double-stranded molecule, which interferes with translation of the mRNA, as the cell will not translate an double-stranded mRNA. Antisense nucleic acids used in the invention are typically at least 10-12 nucleotides in length, for example, at least 15, 20, 25, 50, 75, or 100 nucleotides in length. The antisense nucleic acid can also be as long as the target nucleic acid with which it is

intended that it form an inhibitory duplex. As is described further below, the antisense nucleic acids can be introduced into cells as antisense oligonucleotides, or can be produced in a cell in which a nucleic acid encoding the antisense nucleic acid has been introduced by, for example, using gene therapy methods.

Introduction of FGF-20 antisense nucleic acids into cells affected by a proliferative disorder, for the purpose of gene therapy, can be achieved using a recombinant expression vector, such as a chimeric virus or a colloidal dispersion system, such as a targeted liposome. Those of skill in this art know or can easily ascertain the appropriate route and means for introduction of sense or antisense FGF-20 nucleic acids, without resort to undue experimentation.

Ribozymes are RNA molecules possessing the ability to specifically cleave other single stranded RNA molecules in a manner somewhat analogous to DNA restriction endonucleases. Ribozymes were discovered from the observation that certain mRNAs have the ability to excise their own introns. By modifying the nucleotide sequence of these RNAs, researchers have been able to engineer molecules that recognize specific nucleotide sequences in an RNA molecule and cleave it (Cech, 1988.). Because they are sequence-specific, only mRNAs with particular sequences are inactivated. Investigators have identified two types of ribozymes, Tetrahymena-type and "hammerhead"-type. (Hasselhoff and Gerlach, 1988) Tetrahymena-type ribozymes recognize four-base sequences, while "hammerhead"-type recognize eleven- to eighteen-base sequences. The longer the recognition sequence, the more likely it is to occur exclusively in the target mRNA species. Therefore, hammerhead-type ribozymes are preferable to Tetrahymena-type ribozymes for inactivating a specific mRNA species, and eighteen base recognition sequences are

preferable to shorter recognition sequences. The DNA sequences described herein may thus be used to prepare antisense molecules against, and ribozymes that cleave mRNAs encoding hFGF-20 polypeptides and their ligands directed at decreasing hFGF-20 activity in a mammal.

Gene therapy methods can also be used to deliver genes encoding FGF-20 polypeptides to cells. These methods can be carried out to treat conditions associated with insufficient FGF-20 expression.

In addition to blocking mRNA translation, oligonucleotides, such as antisense oligonucleotides, can be used in methods to stall transcription, such as the triplex method. In this method, an oligonucleotide winds around double-helical DNA in a sequence-specific manner, forming a three-stranded helix, which blocks transcription from the targeted gene. These triplex compounds can be designed to recognize a unique site on a chosen gene (Maher, et al., Antisense Res. and Dev., 1(3):227, 1991; Helene, Anticancer Drug Design, 6(6):569, 1991).

The present invention also provides a composition comprising an isolated hFGF-20 nucleic acid, polypeptide, transgenic mammalian cell, and/or an hFGF-20 epitope binding antibody, and a carrier or diluent.

The present invention also provides a method to induce or inhibit a neurite outgrowth, induce or inhibit neurite adhesion, induce neuronal regeneration, inhibit neuronal degeneration, prevent or reduce frequency and/or severity of seizures, induce or inhibit growth-factor mediated chemotaxis, prevent or treat cell-proliferative disorders, induce or inhibit primary or secondary sexual development, or alter behavioral patterns including, but not limited to, sleep or eating disorders in a mammal wherein said method comprises administering to said mammal a biologically active and pharmaceutically acceptable composition of the present

invention. A preferred embodiment of the method comprises administering to the patient a biologically active and pharmaceutically acceptable composition of the present invention which further comprises at least one other neurotrophic, neuroprotective, thrombolytic, anti-proliferative, and/or anti-thrombotic agent known to be biologically active.

The present invention contemplates a method for treating a mammal suffering from a neurological or cancer condition wherein said method comprises administering to said mammal a biologically active and pharmaceutically acceptable composition of the present invention.

Preferably, the condition to be treated involves one or more of the following disorders: nervous system nerve damage, neurodegeneration, trigeminal neuralgia, glossopharyngeal neuralgia, Bell's Palsy, myasthenia gravis, muscular dystrophy, progressive muscular atrophy, progressive bulbar inherited muscular atrophy, herniated, ruptured or prolapsed invertebrae disk, cervical spondylosis, plexus disorders, thoracic outlet destruction, peripheral neuropathy, such as those caused by lead, dapsone, ticks or porphyria, peripheral myelin disorders, Alzheimer's disease, Gullain-Barre syndrome, Parkinson's disease, Parkinsonian disorders, ALS, multiple sclerosis, other central myelin disorders, stroke, ischemia associated with stroke, neural paropathy, other neural degenerative diseases, motor neuron diseases, sciatic crush, neuropathy associated with diabetes, spinal cord injuries, facial nerve crush, chemotherapy- or pharmacotherapy-induced neuropathy, Huntington's disease, cancer, retinal degenerative diseases, such as retinitis pigmentosa and macular degeneration, and peripheral neuropathies, abnormal primary or secondary sexual development, impotence, infertility, reduced libido, and behavioral disorders such as sleeping or eating

disorders.

A preferred embodiment of the method comprises administering to the patient a biologically active and pharmaceutically acceptable composition of the present invention which further comprises at least one other anti-tumorigenic, neurotrophic, neuroprotective, thrombolytic, anti-proliferative, and/or anti-thrombotic agent known to be biologically active. Most preferably the mammal to be treated is a human.

The methods of the present invention may be particularly useful for treating or preventing neuronal damage resulting from NO mediated toxicity and/or enhancing recovery of neuronal function.

Agonists or antagonists of FGF-20, human or otherwise, as defined herein, intended for administration to a mammal can be formulated into pharmaceutically acceptable compositions for preventing or treating FGF-20 associated conditions or diseases. Such formulations can be dosed in a fashion consistent with good medical practice, taking into account the clinical condition of the individual mammal (especially the side effects of treatment, the site of delivery of the composition, the method of administration, the scheduling of administration, and other factors known to practitioners.

The invention further provides for the use of a FGF-20 agonist or FGF-20 antagonist in the manufacture of a medicament for treating or preventing a neurological disorder, cancer, and/or at least one symptom associated therewith in a mammal.

The pharmaceutical compositions of the present invention maybe administered orally, parenterally, by inhalation spray topically, rectally, nasally, buccally, vaginally or via an implanted reservoir. Preferably, the compositions are administered orally, intraperitoneally or

intravenously. The pharmaceutical compositions of this invention may contain any conventional non-toxic pharmaceutically-acceptable carriers, adjuvants or vehicles. In some cases, the pH of the formulation maybe adjusted with pharmaceutically acceptable acids, bases or buffers to enhance the stability of the formulated compound or its delivery form. Sterile injectable forms of the compositions of this invention may be aqueous or oleaginous suspension. These suspensions may be formulated according to techniques known in the art using suitable dispersion wetting agents and suspending agents. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally acceptable diluent or solvent, for example as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium.

For this purpose, any bland fixed oil may be employed including synthetic mono or di-glycerides. Fatty acids, such as oleic acid and its glyceride derivatives are useful in the preparation of injectables, as are natural pharmaceutically acceptable oils, such as olive oil or castor oil, especially in their polyoxyethylated versions. These oil solutions or suspensions may also contain a long-chain alcohol diluent or dispersant, such as Ph. Helv or similar alcohol.

The pharmaceutical compositions of this invention may be orally administered in any orally acceptable dosage form including, but not limited to, capsules, tablets, aqueous suspensions or solutions. In the case of tablets for oral use, carriers which are commonly used include lactose, corn starch, dicalcium phosphate and microcrystalline cellulose (Avicel). Lubricating agents, such as magnesium stearate

and talc, are also typically added. For oral administration in a capsule form, useful diluents include lactose, dried corn starch and TPGS,as well as the other diluents used in tablets. For oral administration in a soft gelatincapsule form (filled with either a suspension or a solution of a compound of this invention), useful diluents include PEG400, TPGS, propylene glycol, Labrasol, Gelucire, Transcutol, PVP and potassium acetate. When aqueous suspensions are administered orally, the active ingredient is combined with emulsifying and suspending agents,such as sodium CMC, methyl cellulose, pectin and gelatin. If desired, certain sweetening and/or flavoring and/or coloring agents may be added. Alternatively, the pharmaceutical compositions of this invention may be administered in the form of suppositories for rectal administration. These can be prepared by mixing the agent with a suitable nonirritating excipient which is solid at room temperature but liquid at rectal temperature and therefore will melt in the rectum to release the drug. Such materials include cocoa butter, beeswax, gelatin, glycerin and polyethylene glycols.

The pharmaceutical compositions of this invention may also be administered topically, especially when the target of treatment includes areas or organs readily accessible by topical application, including diseases of the eye or the skin. Topically-transdermal patches may also be used. For topical applications, the pharmaceutical compositions may be formulated in a suitable ointment containing the active component suspended or dissolved in one or more carriers.

Carriers for topical administration of the compounds of this invention include, but are not limited to, mineral oil, liquid petrolatum, white petrolatum, propylene glycol, polyoxyethylene, polyoxypropylene compound, emulsifying wax, stearic acid, cetyl stearate, cetyl alcohol, lanolin, magnesium hydroxide, kaolin and water. Alternatively, the

pharmaceutical compositions can be formulated in a suitable lotion or cream containing the active components suspended or dissblved in one or more pharmaceutically acceptable carriers. Suitable carriersinclude, but are not limited to, mineral oil, sorbitan monostearate, polysorbate 60, cetyl esters, wax, cetyl alcohol, 2-octyldodecanol, benzyl alcohol and water.

For ophthalmic use, the pharmaceutical compositions may be formulated as micronized suspensions in isotonic, pH adjusted sterile saline, or, preferably, as solutions in isotonic, pH adjusted sterile saline, either with our without a preservative such as benzylalkonium chloride. Alternatively, for ophthalmic uses, the pharmaceutical compositions may be formulated in an ointment such as petrolatum.

The pharmaceutical compositions of this invention may also be administered by nasal aerosol or inhalation. Such compositions are prepared according to techniques well-known in the art of pharmaceutical formulation and may be prepared as solutions in saline, employing benzyl alcohol or other suitable preservatives, absorption promoters to enhance bioavailability, fluorocarbons, and/or other conventional solubilizing or dispersing agents. The amount of the compound and the amount of any other neurotrophic, neuroprotective, thrombolytic, and/or anti-thrombotic agent, if any, that may be combined with the carrier materials to produce a single dosage form will vary depending upon the host treated and the particular mode of administration.

Preferably, the compositions of this invention should be formulated so that a dosage of between 0.01 - 10 mg/kg body weight/day of a compound of this invention can be administered. More preferably, the dosage is between 0.1 mg/kg body weight/day. In compositions which comprise other neurotrophic, neuroprotective, thrombolytic, and/or anti-

thrombotic agents, said agents and the compounds of the present invention act synergistically or in the least additively to stimulate neurite outgrowth, nerve growth, or neuronal recovery or inhibit neurodegeneration. Therefore, the amount of any other neurotrophic, neuroprotective, thrombolytic, and/or anti-thrombotic agent in such compositions will be less than that required in a monotherapy utilizing only that factor. In such compositions a dosage of between 0.01 - 5 mg/kg body weight/day of each neurotrophic, neuroprotective, thrombolytic, and/or anti-thrombotic agent can be administered in conjunction with a dosage of between 0.01 - 5 mg/kg body weight/day for each hFGF-20 compound comprising the composition. It should also be understood that a specific dosage and treatment regimen for any particular patient will depend upon a variety of factors, including the activity of the specific compound employed, the age, body weight, general health, sex, diet, time of administration, rate of excretion, drug combination, and the judgment of the treating physician and the severity of the particular disease being treated. The amount of active ingredients will also depend upon the particular compound of this invention and the neurotrophic, neuroprotective, thrombolytic, and/or anti-thrombotic agents used in the pharmaceutical composition.

A preferred embodiment of this aspect of the invention includes administering the FGF-20 compound and any other neurotrophic, neuroprotective, thrombolytic, and/or anti-thrombotic agent in a single dosage form when they are to be co-administered.

In another aspect of this embodiment, the pharmaceutical compositions of the present invention can be used in a method to stimulate nerve growth ex vivo. For this aspect, the compounds or compositions described above

can be applied directly to the nerve cells in culture. This aspect of the invention is useful for *ex vivo* nerve regeneration.

The compositions of the present invention are also useful in bioassays measuring neurite outgrowth or neuronal migration altering activities. For example, the examples described herein (see in particular, Example 6 and 7) can be modified such that cell lines or cortical explants are co-cultured with cells expressing hFGF-20 polypeptides or fragments thereof. If desirable, a similar assay can be performed to identify small molecules (including hFGF-20 peptides or chemical molecules) that block hFGF-20's growth promoting effects. hFGF-20 may act as "kindling" in disorders such as epilepsy, by contributing to sustained and increased excitability of dendrites through its neurite outgrowth promoting activity. Consequently, it would be desirable to block hFGF-20's growth promoting effects in such disorders. Further, blocking hFGF-20's migration promoting activity might be useful for treatment of various neuronal tumors including, but not limited to, glial cell tumors. The desired biological function may be obtained from polypeptides ranging in size from fragments containing a single epitope to which an antibody molecule can bind to large polypeptides which are capable of participating in the characteristic induction or programming of phenotypic changes within a cell.

Gene Therapy

Nucleic acids encoding hFGF-20 polypeptides may also be used in gene therapy. In gene therapy applications, genes are introduced into cells in order to achieve in vivo synthesis of a therapeutically effective genetic product, for example, for replacement of a defective gene. "Gene therapy" includes both conventional gene therapy where a

lasting effect is achieved by a single treatment, and the administration of gene therapeutic agents, which involves the one time or repeated administration of a therapeutically effective DNA or mRNA.

Gene transfer systems known in the art may be useful in the practice of the gene therapy methods of the present invention. These include viral and nonviral transfer methods. A number of viruses have been used as gene transfer vectors, including papovaviruses (e.g., SV40, Madzak et al., 1992), adenovirus (Berkner, 1992; Berkner et al., 1988; Gorziglia and Kapikian, 1992; Quantin et al., 1992; Rosenfeld et al., 1992; Wilkinson et al., 1992; Stratford-Perricaudet et al., 1990), vaccinia virus (Moss, 1992), adeno-associated virus (Muzyczka, 1992; Ohi et al., 1990), herpesviruses including HSV and EBV (Margolskee, 1992; Johnson et al., 1992; Fink et al., 1992; Breakfield and Geller, 1987; Freese et al., 1990), and retroviruses of avian (Brandyopadhyay and Temin, 1984; Petropoulos et al., 1992), murine (Miller, 1992; Miller et al., 1985; Sorge et al., 1984; Mann and Baltimore, 1985; Miller et al., 1988), and human origin (Shimada et al., 1991; Helseth et al., 1990; Page et al., 1990; Buchschacher and Panganiban, 1992). Most human gene therapy protocols have been based on disabled murine retroviruses.

Nonviral gene transfer methods known in the art include chemical techniques such as calcium phosphate coprecipitation (Graham and van der Eb, 1973; Pellicer et al., 1980); mechanical techniques, for example microinjection (Anderson et al., 1980; Gordon et al., 1980; Brinster et al., 1981; Constantini and Lacy, 1981); membrane fusion-mediated transfer via liposomes (Felgner et al., 1987; Wang and Huang, 1989; Kaneda et al, 1989; Stewart et al., 1992; Nabel et al., 1990; Lim et al., 1992); and direct DNA uptake and receptor-mediated DNA transfer (Wolff et al.,

1990; Wu et al., 1991; Zenke et al., 1990; Wu et al., 1989b; Wolff et al., 1991; Wagner et al., 1990; Wagner et al., 1991; Cotten et al., 1990; Curiel et al., 1991a; Curiel et al., 1991b). Viral-mediated gene transfer can be combined with direct in vivo gene transfer using liposome delivery, allowing one to direct the viral vectors to the tumor cells and not into the surrounding nondividing cells. Alternatively, the retroviral vector producer cell line can be injected into tumors (Culver et al., 1992). Injection of producer cells would then provide a continuous source of vector particles. This technique has been approved for use in humans with inoperable brain tumors.

As mentioned previously antisense RNAs and DNAs can be used as therapeutic agents for blocking the expression of certain genes in vivo. It has already been shown that short antisense oligonucleotides can be imported into cells where act as inhibitors, despite their low intracellular concentrations caused by their restricted uptake by the cell membrane. Zamecnik et al., Proc. Natl. Acad Sci. USA 83: 4143-4146 [1986]). The oligonucleotides can be modified to enhance their uptake, e.g., by substituting their negatively charged phosphodiester groups by uncharged groups. There are a variety of techniques available for introducing nucleic acids into viable cells. The techniques vary depending upon whether the nucleic acid is transferred into cultured cell in vitro, or in vivo in the cells of the intended host. Techniques suitable for the transfer of nucleic acid into mammalian cells in vitro include the use of liposomes, electroporation, micro-injection, cell fusion, DEAE-dextran, the calcium phosphate precipitation method, etc.

The currently preferred in vivo gene transfer techniques include transfection with viral (typically retroviral) vectors and viral coat protein-liposome mediated transfection (Dzau, et al., Trends in Biotechnology 11: 205-

210 (1991). Liposome/DNA complexes have been shown to be capable of mediating direct in vivo gene transfer. While in standard liposome preparations the gene transfer process is nonspecific, localized in vivo uptake and expression have been reported in tumor deposits, for example, following direct in situ administration (Nabel, 1992). In some situations it is desirable to provide the nucleic acid source with an agent that targets the target cells, such as an antibody specific for a cell surface membrane protein or the target cell, a ligand for a receptor on the target cells, etc. When liposomes are employed, proteins which bind to a cell surface membrane protein associated with endocytosis may by used for targeting and/or to facilitate uptake, e.g., capsid proteins or fragments thereof tropic for a particular cell type, antibodies for proteins which undergo internalization in cycling, protein that target intracellular localization and enhance intracellular half-life. The technique of receptor-mediated endocytosis is described, for example by Wu et al., J. Biol. Chem. 262: 4429-4432 (1987). For a review of gene marking and gene therapy protocols see Anderson et al., Science 256: 808-813 (1992).

Having generally described the invention, the same will be more readily understood by reference to the following examples, which are provided by way of illustration and are not intended as limiting.

EXAMPLES

Example 1: Expression and Purification of an hFGF-20 Polypeptide in E. coli

The bacterial expression vector pQE60 is used for bacterial expression in this example. (QIAGEN, Inc., Chatsworth, CA). pQE60 encodes ampicillin antibiotic resistance ("Ampr") and contains a bacterial origin of

replication ("ori"), an IPTG inducible promoter, a ribosome binding site ("RBS"), six codons encoding histidine residues that allow affinity purification using nickel-nitrilo-tri-acetic acid ("Ni-NTA") affinity resin sold by QIAGEN, Inc., and suitable single restriction enzyme cleavage sites. These elements are arranged such that a DNA fragment encoding a polypeptide can be inserted in such a way as to produce that polypeptide with the six His residues (i.e., a "6 X His tag") covalently linked to the carboxyl terminus of that polypeptide. However, a polypeptide coding sequence can optionally be inserted such that translation of the six His codons is prevented and, therefore, a polypeptide is produced with no 6 X His tag.

The nucleic acid sequence encoding the desired portion of an hFGF-20 polypeptide lacking the hydrophobic leader sequence is amplified from the deposited cDNA clone using PCR oligonucleotide primers (based on the sequences presented, e.g., as presented in at least one of SEQ ID NOS:1 OR 2), which anneal to the amino terminal encoding DNA sequences of the desired portion of an hFGF-20 polypeptide and to sequences in the deposited construct 3' to the cDNA coding sequence. Additional nucleotides containing restriction sites to facilitate cloning in the pQE60 vector are added to the 5' and 3' sequences, respectively.

For cloning an hFGF-20 polypeptide, the 5' and 3' primers have nucleotides corresponding or complementary to a portion of the coding sequence of an hFGF-20, e.g., as presented in at least one of SEQ ID NOS:1, 2, or 3, according to known method steps. One of ordinary skill in the art would appreciate, of course, that the point in a polypeptide coding sequence where the 5' primer begins can be varied to amplify a desired portion of the complete polypeptide shorter or longer than the mature form.

The amplified hFGF-20 nucleic acid fragments and the vector pQE60 are digested with appropriate restriction enzymes and the digested DNAs are then ligated together. Insertion of the hFGF-20 DNA into the restricted pQE60 vector places an hFGF-20 polypeptide coding region including its associated stop codon downstream from the IPTG-inducible promoter and in-frame with an initiating AUG codon. The associated stop codon prevents translation of the six histidine codons downstream of the insertion point.

The ligation mixture is transformed into competent E. coli cells using standard procedures such as those described in Sambrook, et al., 1989; Ausubel, 1987-1998. E. coli strain M15/rep4, containing multiple copies of the plasmid pREP4, which expresses the lac repressor and confers kanamycin resistance ("Kanr"), is used in carrying out the illustrative example described herein. This strain, which is only one of many that are suitable for expressing hFGF-20 polypeptide, is available commercially from QIAGEN, Inc. Transformants are identified by their ability to grow on LB plates in the presence of ampicillin and kanamycin. Plasmid DNA is isolated from resistant colonies and the identity of the cloned DNA confirmed by restriction analysis, PCR and DNA sequencing.

Clones containing the desired constructs are grown overnight ("O/N") in liquid culture in LB media supplemented with both ampicillin (100 µg/ml) and kanamycin (25 µg/ml). The O/N culture is used to inoculate a large culture, at a dilution of approximately 1:25 to 1:250. The cells are grown to an optical density at 600 nm ("OD600") of between 0.4 and 0.6. Isopropyl-b-D-thiogalactopyranoside ("IPTG") is then added to a final concentration of 1 mM to induce transcription from the lac repressor sensitive promoter, by inactivating the lacI repressor. Cells subsequently are

incubated further for 3 to 4 hours. Cells then are harvested by centrifugation.

The cells are then stirred for 3-4 hours at 4°C in 6M guanidine-HCl, pH8. The cell debris is removed by centrifugation, and the supernatant containing the hFGF-20 is dialyzed against 50 mM Na-acetate buffer pH6, supplemented with 200 mM NaCl. Alternatively, a polypeptide can be successfully refolded by dialyzing it against 500 mM NaCl, 20% glycerol, 25 mM Tris/HCl pH7.4, containing protease inhibitors.

If insoluble protein is generated, the protein is made soluble according to known method steps. After renaturation the polypeptide is purified by ion exchange, hydrophobic interaction and size exclusion chromatography. Alternatively, an affinity chromatography step such as an antibody column is used to obtain pure hFGF-20 polypeptide. The purified polypeptide is stored at 4°C or frozen at -40°C to -120°C.

Example 2: Cloning and Expression of an hFGF-20 Polypeptide in a Baculovirus Expression System

As an illustrative example, the plasmid shuttle vector pA2 GP is used to insert the cloned DNA encoding the mature polypeptide into a baculovirus to express an hFGF-20 polypeptide, using a baculovirus leader and standard methods as described in Summers, et al., A Manual of Methods for Baculovirus Vectors and Insect Cell Culture Procedures, Texas Agricultural Experimental Station Bulletin No. 1555 (1987). This expression vector contains the strong polyhedrin promoter of the Autographa californica nuclear polyhedrosis virus (AcMNPV) followed by the secretory signal peptide (leader) of the baculovirus gp67 polypeptide and convenient restriction sites such as BamHI, Xba I and Asp718. The polyadenylation site of the simian virus 40

("SV40") is used for efficient polyadenylation. For easy selection of recombinant virus, the plasmid contains the beta-galactosidase gene from E. coli under control of a weak Drosophila promoter in the same orientation, followed by the polyadenylation signal of the polyhedrin gene. The inserted genes are flanked on both sides by viral sequences for cell-mediated homologous recombination with wild-type viral DNA to generate viable virus that expresses the cloned polynucleotide.

Other baculovirus vectors can be used in place of the vector above, such as pAc373, pVL941 and pAcIM1, as one skilled in the art would readily appreciate, as long as the construct provides appropriately located signals for transcription, translation, secretion and the like, including a signal peptide and an in-frame AUG as required. Such vectors are described, for instance, in Luckow, et al., Virology 170:31-39.

The cDNA sequence encoding the mature hFGF-20 polypeptide in the deposited or other clone, lacking the AUG initiation codon and the naturally associated nucleotide binding site, is amplified using PCR oligonucleotide primers corresponding to the 5' and 3' sequences of the gene. Non-limiting examples include 5' and 3' primers having nucleotides corresponding or complementary to a portion of the coding sequence of an hFGF-20 polypeptide, e.g., as presented in at least one of SEQ ID NOS:1, 2, or 3 according to known method steps.

The amplified fragment is isolated from a 1% agarose gel using a commercially available kit (e.g., "Geneclean," BIO 101 Inc., La Jolla, CA). The fragment then is then digested with the appropriate restriction enzyme and again is purified on a 1% agarose gel. This fragment is designated herein "F1".

The plasmid is digested with the corresponding restriction enzymes and optionally, can be dephosphorylated using calf intestinal phosphatase, using routine procedures known in the art. The DNA is then isolated from a 1% agarose gel using a commercially available kit ("Geneclean" BIO 101 Inc., La Jolla, CA). This vector DNA is designated herein "V1".

Fragment F1 and the dephosphorylated plasmid V1 are ligated together with T4 DNA ligase. E. coli HB101 or other suitable E. coli hosts such as XL-1 Blue (Stratagene Cloning Systems, La Jolla, CA) cells are transformed with the ligation mixture and spread on culture plates. Bacteria that contain the plasmid with the human hFGF-20 gene are identified using a PCR method in which one of the primers that is used is designed to amplify the gene and the second primer is from well within the vector so that only those bacterial colonies containing the hFGF-20 gene fragment will show amplification of the DNA. The sequence of the cloned fragment is confirmed by DNA sequencing. This plasmid is designated herein pBacFGF-20 .

Five μg of the plasmid pBachFGF-20 is co-transfected with 1.0 μg of a commercially available linearized baculovirus DNA ("BaculoGold™ baculovirus DNA", Pharmingen, San Diego, CA), using the lipofection method described by Felgner, et al., Proc. Natl. Acad. Sci. USA 84:7413-7417 (1987). 1 μg of BaculoGold™ virus DNA and 5 μg of the plasmid pBacFGF-20 are mixed in a sterile well of a microtiter plate containing 50 μl of serum-free Grace's medium (Life Technologies, Inc., Rockville, MD). Afterwards, 10 μl Lipofectin plus 90 μl Grace's medium are added, mixed and incubated for 15 minutes at room temperature. Then the transfection mixture is added drop-wise to Sf9 insect cells (ATCC CRL 1711) seeded in a 35 mm

tissue culture plate with 1 ml Grace's medium without serum. The plate is rocked back and forth to mix the newly added solution. The plate is then incubated for 5 hours at 27°C. After 5 hours the transfection solution is removed from the plate and 1 ml of Grace's insect medium supplemented with 10% fetal calf serum is added. The plate is put back into an incubator and cultivation is continued at 27°C for four days.

After four days the supernatant is collected and a plaque assay is performed, according to known methods. An agarose gel with "Blue Gal" (Life Technologies, Inc., Rockville, MD) is used to allow easy identification and isolation of gal-expressing clones, which produce blue-stained plaques. (A detailed description of a "plaque assay" of this type can also be found in the user's guide for insect cell culture and baculovirology distributed by Life Technologies, Inc., Rockville, MD, page 9-10). After appropriate incubation, blue stained plaques are picked with a micropipettor tip (e.g., Eppendorf). The agar containing the recombinant viruses is then resuspended in a microcentrifuge tube containing 200 µl of Grace's medium and the suspension containing the recombinant baculovirus is used to infect Sf9 cells seeded in 35 mm dishes. Four days later the supernatants of these culture dishes are harvested and then they are stored at 4°C. The recombinant virus is called V-hFGF-20 .

To verify the expression of the hFGF-20 gene, Sf9 cells are grown in Grace's medium supplemented with 10% heat-inactivated FBS. The cells are infected with the recombinant baculovirus V-hFGF-20 at a multiplicity of infection ("MOI") of about 2. Six hours later the medium is removed and is replaced with SF900 II medium minus methionine and cysteine (available, e.g., from Life Technologies, Inc., Rockville, MD). If radiolabeled

polypeptides are desired, 42 hours later, 5 mCi of 35S-methionine and 5 mCi 35S-cysteine (available from Amersham) are added. The cells are further incubated for 16 hours and then they are harvested by centrifugation. The polypeptides in the supernatant as well as the intracellular polypeptides are analyzed by SDS-PAGE followed by autoradiography (if radiolabeled). Microsequencing of the amino acid sequence of the amino terminus of purified polypeptide can be used to determine the amino terminal sequence of the mature polypeptide and thus the cleavage point and length of the secretory signal peptide.

Example 3: Cloning and Expression of hFGF-20 in Mammalian Cells

A typical mammalian expression vector contains at least one promoter element, which mediates the initiation of transcription of mRNA, the polypeptide coding sequence, and signals required for the termination of transcription and polyadenylation of the transcript. Additional elements include enhancers, Kozak sequences and intervening sequences flanked by donor and acceptor sites for RNA splicing. Highly efficient transcription can be achieved with the early and late promoters from SV40, the long terminal repeats (LTRS) from Retroviruses, e.g., RSV, HTLVI, HIVI and the early promoter of the cytomegalovirus (CMV). However, cellular elements can also be used (e.g., the human actin promoter). Suitable expression vectors for use in practicing the present invention include, for example, vectors such as pIRES1neo, pRetro-Off, pRetro-On, PLXSN, or pLNCX (Clonetech Labs, Palo Alto, CA), pcDNA3.1 (+/-), pcDNA/Zeo (+/-) or pcDNA3.1/Hygro (+/-) (Invitrogen), PSVL and PMSG (Pharmacia, Uppsala, Sweden), pRSVcat (ATCC 37152), pSV2dhfr (ATCC 37146) and pBC12MI (ATCC 67109). Mammalian host cells that could be used include human Hela 293, H9 and

Jurkat cells, mouse NIH3T3 and C127 cells, Cos 1, Cos 7 and CV 1, quail QC1-3 cells, mouse L cells and Chinese hamster ovary (CHO) cells.

Alternatively, the gene can be expressed in stable cell lines that contain the gene integrated into a chromosome. The co-transfection with a selectable marker such as dhfr, gpt, neomycin, or hygromycin allows the identification and isolation of the transfected cells.

The transfected gene can also be amplified to express large amounts of the encoded polypeptide. The DHFR (dihydrofolate reductase) marker is useful to develop cell lines that carry several hundred or even several thousand copies of the gene of interest. Another useful selection marker is the enzyme glutamine synthase (GS) (Murphy, et al., Biochem. J. 227:277-279 (1991); Bebbington, et al., Bio/Technology 10:169-175 (1992)). Using these markers, the mammalian cells are grown in selective medium and the cells with the highest resistance are selected. These cell lines contain the amplified gene(s) integrated into a chromosome. Chinese hamster ovary (CHO) and NSO cells are often used for the production of polypeptides.

The expression vectors pC1 and pC4 contain the strong promoter (LTR) of the Rous Sarcoma Virus (Cullen, et al., Molec. Cell. Biol. 5:438-447 (1985)) plus a fragment of the CMV-enhancer (Boshart, et al., Cell 41:521-530 (1985)). Multiple cloning sites, e.g., with the restriction enzyme cleavage sites BamHI, XbaI and Asp718, facilitate the cloning of the gene of interest. The vectors contain in addition the 3' intron, the polyadenylation and termination signal of the rat preproinsulin gene.

Example 3(a): Cloning and Expression in COS Cells

The expression plasmid, phFGF-20HA, is made by cloning a cDNA encoding hFGF-20 into the expression vector

pcDNAI/Amp or pcDNAIII (which can be obtained from Invitrogen, Inc.). The expression vector pcDNAI/amp contains: (1) an E. coli origin of replication effective for propagation in E. coli and other prokaryotic cells; (2) an ampicillin resistance gene for selection of plasmid-containing prokaryotic cells; (3) an SV40 origin of replication for propagation in eucaryotic cells; (4) a CMV promoter, a polylinker, an SV40 intron; (5) several codons encoding a hemagglutinin fragment (i.e., an "HA" tag to facilitate purification) or HIS tag (see, e.g, Ausubel, supra) followed by a termination codon and polyadenylation signal arranged so that a cDNA can be conveniently placed under expression control of the CMV promoter and operably linked to the SV40 intron and the polyadenylation signal by means of restriction sites in the polylinker. The HA tag corresponds to an epitope derived from the influenza hemagglutinin polypeptide described by Wilson, et al., Cell 37:767-778 (1984). The fusion of the HA tag to the target polypeptide allows easy detection and recovery of the recombinant polypeptide with an antibody that recognizes the HA epitope. pcDNAIII contains, in addition, the selectable neomycin marker.

A DNA fragment encoding the hFGF-20 is cloned into the polylinker region of the vector so that recombinant polypeptide expression is directed by the CMV promoter. The plasmid construction strategy is as follows. The hFGF-20 cDNA of the deposited clone is amplified using primers that contain convenient restriction sites, much as described above for construction of vectors for expression of hFGF-20 in E. coli. Non-limiting examples of suitable primers include those based on the coding sequences presented in at least one of SEQ ID NOS:1 OR 2, as they encode hFGF-20 polypeptides as described herein.

The PCR amplified DNA fragment and the vector, pcDNAI/Amp, are digested with suitable restriction enzyme(s) and then ligated. The ligation mixture is transformed into E. coli strain SURE (available from Stratagene Cloning Systems, 11099 North Torrey Pines Road, La Jolla, CA 92037), and the transformed culture is plated on ampicillin media plates which then are incubated to allow growth of ampicillin resistant colonies. Plasmid DNA is isolated from resistant colonies and examined by restriction analysis or other means for the presence of the hFGF-20 -encoding fragment.

For expression of recombinant hFGF-20, COS cells are transfected with an expression vector, as described above, using DEAE-DEXTRAN, as described, for instance, in Sambrook, et al., Molecular Cloning: a Laboratory Manual, Cold Spring Laboratory Press, Cold Spring Harbor, New York (1989). Cells are incubated under conditions for expression of hFGF-20 by the vector.

Expression of the hFGF-20 -HA fusion polypeptide is detected by radio-labeling and immuno-precipitation, using methods described in, for example Harlow, et al., Antibodies: A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (1988). To this end, two days after transfection, the cells are labeled by incubation in media containing 35S-cysteine for 8 hours. The cells and the media are collected, and the cells are washed and lysed with detergent-containing RIPA buffer: 150 mM NaCl, 1% NP-40, 0.1% SDS, 0.5% DOC, 50 mM TRIS, pH 7.5, as described by Wilson, et al. cited above. Proteins are precipitated from the cell lysate and from the culture media using an HA-specific monoclonal antibody. The precipitated polypeptides then are analyzed by SDS-PAGE and autoradiography. An expression product of the expected size

is seen in the cell lysate, which is not seen in negative controls.

Example 3(b): Cloning and Expression in CHO Cells

The vector pC4 is used for the expression of hFGF-20 polypeptide. Plasmid pC4 is a derivative of the plasmid pSV2-dhfr (ATCC Accession No. 37146). The plasmid contains the mouse DHFR gene under control of the SV40 early promoter. Chinese hamster ovary- or other cells lacking dihydrofolate activity that are transfected with these plasmids can be selected by growing the cells in a selective medium (alpha minus MEM, Life Technologies) supplemented with the chemotherapeutic agent methotrexate. The amplification of the DHFR genes in cells resistant to methotrexate (MTX) has been well documented (see, e.g., F. W. Alt, et al., J. Biol. Chem. 253:1357-1370 (1978); J. L. Hamlin and C. Ma, Biochem. et Biophys. Acta 1097:107-143 (1990); and M. J. Page and M. A. Sydenham, Biotechnology 9:64-68 (1991)). Cells grown in increasing concentrations of MTX develop resistance to the drug by overproducing the target enzyme, DHFR, as a result of amplification of the DHFR gene. If a second gene is linked to the DHFR gene, it is usually co-amplified and over-expressed. It is known in the art that this approach can be used to develop cell lines carrying more than 1,000 copies of the amplified gene(s). Subsequently, when the methotrexate is withdrawn, cell lines are obtained which contain the amplified gene integrated into one or more chromosome(s) of the host cell.

Plasmid pC4 contains for expressing the gene of interest the strong promoter of the long terminal repeat (LTR) of the Rous Sarcoma Virus (Cullen, et al., Molec. Cell. Biol. 5:438-447 (1985)) plus a fragment isolated from the enhancer of the immediate early gene of human cytomegalovirus (CMV) (Boshart, et al., Cell 41:521-530 (1985)). Downstream of the promoter are BamHI, XbaI, and

Asp718 restriction enzyme cleavage sites that allow integration of the genes. Behind these cloning sites the plasmid contains the 3' intron and polyadenylation site of the rat preproinsulin gene. Other high efficiency promoters can also be used for the expression, e.g., the human b-actin promoter, the SV40 early or late promoters or the long terminal repeats from other retroviruses, e.g., HIV and HTLVI. Clontech's Tet-Off and Tet-On gene expression systems and similar systems can be used to express the hFGF-20 in a regulated way in mammalian cells (M. Gossen, and H. Bujard, Proc. Natl. Acad. Sci. USA 89: 5547-5551 (1992)). For the polyadenylation of the mRNA other signals, e.g., from the human growth hormone or globin genes can be used as well. Stable cell lines carrying a gene of interest integrated into the chromosomes can also be selected upon co-transfection with a selectable marker such as gpt, G418 or hygromycin. It is advantageous to use more than one selectable marker in the beginning, e.g., G418 plus methotrexate.

The plasmid pC4 is digested with restriction enzymes and then dephosphorylated using calf intestinal phosphatase by procedures known in the art. The vector is then isolated from a 1% agarose gel.

The DNA sequence encoding the complete hFGF-20 polypeptide is amplified using PCR oligonucleotide primers corresponding to the 5' and 3' sequences of the gene. Non-limiting examples include 5' and 3' primers having nucleotides corresponding or complementary to a portion of the coding sequence of an hFGF-20 , e.g., as presented in at least one of SEQ ID NOS:1 OR 2, according to known method steps.

The amplified fragment is digested with suitable endonucleases and then purified again on a 1% agarose gel. The isolated fragment and the dephosphorylated vector are

then ligated with T4 DNA ligase. E. coli HB101 or XL-1 Blue cells are then transformed and bacteria are identified that contain the fragment inserted into plasmid pC4 using, for instance, restriction enzyme analysis.

Chinese hamster ovary (CHO) cells lacking an active DHFR gene are used for transfection. 5 µg of the expression plasmid pC4 is cotransfected with 0.5 µg of the plasmid pSV2-neo using lipofectin. The plasmid pSV2neo contains a dominant selectable marker, the neo gene from Tn5 encoding an enzyme that confers resistance to a group of antibiotics including G418. The cells are seeded in alpha minus MEM supplemented with 1 µg/ml G418. After 2 days, the cells are trypsinized and seeded in hybridoma cloning plates (Greiner, Germany) in alpha minus MEM supplemented with 10, 25, or 50 ng/ml of methotrexate plus 1 µg/ml G418. After about 10-14 days single clones are trypsinized and then seeded in 6-well petri dishes or 10 ml flasks using different concentrations of methotrexate (50 nM, 100 nM, 200 nM, 400 nM, 800 nM). Clones growing at the highest concentrations of methotrexate are then transferred to new 6-well plates containing even higher concentrations of methotrexate (1 mM, 2 mM, 5 mM, 10 mM, 20 mM). The same procedure is repeated until clones are obtained which grow at a concentration of 100 - 200 mM. Expression of the desired gene product is analyzed, for instance, by SDS-PAGE and Western blot or by reverse phase HPLC analysis.

Example 4: Tissue Distribution of hFGF-20 mRNA Expression

Northern blot analysis is carried out to examine hFGF-20 gene expression in human tissues, using methods described by, among others, Sambrook, et al., cited above. A cDNA probe containing the entire nucleotide sequence of an hFGF-20 polypeptide (SEQ ID NOS:1) is labeled with ^{32}P using the

Rediprime™ DNA labeling system (Amersham Life Science), according to the manufacturer's instructions. After labeling, the probe is purified using a CHROMA SPIN-100™ column (Clontech Laboratories, Inc.), according to the manufacturer's protocol number PT1200-1. The purified and labeled probe is used to examine various human tissues for hFGF-20 mRNA.

Multiple Tissue Northern (MTN) blots containing various human tissues (H) or human immune system tissues (IM) are obtained from Clontech and are examined with the labeled probe using ExpressHyb hybridization solution (Clontech) according to manufacturer's protocol number PT1190-1. Following hybridization and washing, the blots are mounted and exposed to film at -70 °C overnight, and films developed according to standard procedures. The results show hFGF-20 polypeptides to be selectively expressed in neuronal tissues.

EXAMPLE 5: Directed Mutagenesis of hFGF-20 Polypeptides to Provide DNA Encoding Specified Substitutions, Insertions or Deletions of SEQ ID NO:1 using the Polymerase Chain Reaction

The polymerase chain reaction (PCR) can be used for the enzymatic amplification and direct sequencing of small quantities of nucleic acids (see, e.g., Ausubel, supra, section 15) to provide specified substitutions, insertions or deletions in DNA encoding an hFGF-20 polypeptide of the present inventions, e.g., SEQ ID NO:1, 2, 3, or any sequence described herein, as presented herein, to provide an hFGF-20 polypeptide sequence of interest including at least one substitution, insertion or deletion selected from the group consisting of 5G, 9N, 10Y, 11F, 13V, 14Q, 15D, 16A, 17V, D18, D19, 20P, 21F, 23N, 24V, 25P, 26V, 29V, 50R, 53A, 54V, 55T, 56D, 58D, 61K, 78E, 80F, 82N, 84T, 85I, 90K, 94R, 103I, 123E, 135Q, 138V, 155L, 159V, 166Y, 177E, 179T, 181T, 198D,

199K, 207I, 209S, 210Q, and 211S of SEQ ID NO:4 or the corresponding amino acid of SEQ ID NO:5. This technology can be used as a quick and efficient method for introducing any desired sequence change into the DNA of interest.

Unit 8.5 of Ausubel, supra, contains two basic protocols for introducing base changes into specific DNA sequences. Basic Protocol 1, as presented in the first section 8.5 of Ausubel, supra (entirely incorporated herein by reference), describes the incorporation of a restriction site and Basic Protocol 2, as presented below and in the second section of Unit 8.5 of Ausubel, supra, details the generation of specific point mutations (all of the following references in this example are to sections of Ausubel et al., eds., Current Protocols in Molecular Biology, Wiley Interscience, New York (1987-1999)). An alternate protocol describes generating point mutations by sequential PCR steps. Although the general procedure is the same in all three protocols, there are differences in the design of the synthetic oligonucleotide primers and in the subsequent cloning and analyses of the amplified fragments.

The PCR procedure described here can rapidly, efficiently, and/or reproducibly introduce any desired change into a DNA fragment. It is similar to the oligonucleotide-directed mutagenesis method described in UNIT 8.1, but does not require the preparation of a uracil-substituted DNA template.

The main disadvantage of PCR-generated mutagenesis is related to the fidelity of the Taq DNA polymerase. The mutation frequency for Taq DNA polymerase was initially estimated to be as high as 1/5000 per cycle (Saiki et al., 1988). This means that the entire amplified fragment must be sequenced to be sure that there are no Taq-derived mutations. To reduce the amount of sequencing required, it is best to introduce the mutation by amplifying as small a

fragment as possible. With rapid and reproducible methods of double-stranded DNA sequencing (UNIT 7.4), the entire amplified fragment can usually be sequenced from a single primer. If the fragment is somewhat longer, it is best to subclone the fragment into an M13-derived vector, so that both forward and reverse primers can be used to sequence the amplified fragment.

If there are no convenient restriction sites flanking the fragment of interest, the utility of this method is somewhat reduced. Many researchers prefer the mutagenesis procedure in UNIT 8.1 to avoid excessive sequencing.

A full discussion of critical parameters for PCR amplification can be found in UNIT 15.1. (see also, Saiki, R.K., Gelfand, D.H., Stoffel, S., Scharf, S.J., Higuchi, R., Horn, G.T., Mullis, K.B., and Erlich, H.A. 1988. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science 239:487-491).

BASIC PROTOCOL (2): POINT MUTATIONS BY PCR

In this protocol, synthetic oligonucleotides are designed to incorporate a point mutation at one end of an amplified fragment. Following PCR, the amplified fragments are made blunt-ended by treatment with Klenow fragment. These fragments are then ligated and subcloned into a vector to facilitate sequence analysis. This procedure is summarized in Figure 8.5.2 of Ausubel, supra. Required materials include the DNA sample to be mutagenized, klenow fragment of E. coli DNA polymerase I (UNIT 3.5 of Ausubel, supra), appropriate restriction endonucleases (Table 8.5.1), as well as, the reagents and equipment for synthesis, purification, and phosphorylation of oligonucleotides (UNITS 2.11, 2.12, & 3.10), electrophoresis on nondenaturing agarose and low gelling/melting agarose gels (UNITS 2.5A & 2.6), ligation of DNA fragments (UNIT 3.16), transformation

of E. coli (UNIT 1.8), and preparation of plasmid DNA (UNIT 1.6).

Prepare template DNA (see Basic Protocol 1, steps 1 and 2). Synthesize (UNIT 2.11) and purify (UNIT 2.12) the oligonucleotide primers (primers 3 and 4 in Fig. 8.5.2B) accordingly. The oligonucleotide primers must be homologous to the template DNA for more than 15 bases. No four-base "clamp" sequence is added to these primers. The primer sequences are based on a DNA encoding the hFGF-20 polypeptide sequence of interest including at least one substitution, insertion or deletion selected from the group consisting of 5G, 9N, 10Y, 11F, 13V, 14Q, 15D, 16A, 17V, D18, D19, 20P, 21F, 23N, 24V, 25P, 26V, 29V, 50R, 53A, 54V, 55T, 56D, 58D, 61K, 78E, 80F, 82N, 84T, 85I, 90K, 94R, 103I, 123E, 135Q, 138V, 155L, 159V, 166Y, 177E, 179T, 181T, 198D, 199K, 207I, 209S, 210Q, and 211S of SEQ ID NO:4 or the corresponding amino acid of SEQ ID NO:5. Phosphorylate the 5' end of the oligonucleotides (UNIT 3.10). This step is necessary because the 5' end of the oligonucleotide will be used directly in cloning.

Amplify the template DNA (see Basic Protocol 1, steps 5 and 6). After the final extension step, add 5 U Klenow fragment to the reaction mix and incubate 15 min at 30 °C. During PCR, the Taq polymerase adds an extra nontemplated nucleotide to the 3' end of the fragment. The 3'-5' exonuclease activity of the Klenow fragment is required to make the ends flush and suitable for blunt-end cloning (UNIT 3.5). Analyze and process the reaction mix (see Basic Protocol 1, steps 7 and 8). Digest half the amplified fragments with the restriction endonucleases for the flanking sequences (UNIT 3.1). Purify digested fragments on a low gelling/melting agarose gel (UNIT 2.6).

Subclone the two amplified fragments into an appropriately digested vector by blunt-end ligation (UNIT

3.16). Transform recombinant plasmid into E. coli (UNIT 1.8). Prepare DNA by plasmid miniprep (UNIT 1.6). Analyze the amplified fragment portion of the plasmid DNA by DNA sequencing to confirm the point mutation (UNIT 7.4). This is critical because the Taq DNA polymerase can introduce additional mutations into the fragment (see Critical Parameters).

ALTERNATE PROTOCOL: POINT MUTATION BY SEQUENTIAL PCR

In this procedure, the two fragments encompassing the mutation are annealed with each other and extended by mutually primed synthesis; this fragment is then amplified by a second PCR step, thereby avoiding the blunt-end ligation required in Basic Protocol 2. This strategy is outlined in Figure 8.5.3. For materials, see Basic Protocols 1 and 2 of Ausubel, supra.

Prepare template DNA (see Basic Protocol 1, steps 1 and 2). Synthesize (UNIT 2.11) and purify (UNIT 2.12) the oligonucleotide primers (primers 5 and 6 in Fig. 8.5.3B) to generate an hFGF-20 polypeptide sequence of interest including at least one substitution, insertion or deletion selected from the group consisting of 5G, 9N, 10Y, 11F, 13V, 14Q, 15D, 16A, 17V, D18, D19, 20P, 21F, 23N, 24V, 25P, 26V, 29V, 50R, 53A, 54V, 55T, 56D, 58D, 61K, 78E, 80F, 82N, 84T, 85I, 90K, 94R, 103I, 123E, 135Q, 138V, 155L, 159V, 166Y, 177E, 179T, 181T, 198D, 199K, 207I, 209S, 210Q, and 211S of SEQ ID NO:4 or the corresponding amino acid of SEQ ID NO:5. The oligonucleotides must be homologous to the template for 15 to 20 bases and must overlap with one another by at least 10 bases. The 5' end does not have a "clamp" sequence.

Amplify the template DNA and generate blunt-end fragments (see Basic Protocol 2, steps 4 and 5). Purify the fragments by nondenaturing agarose gel electrophoresis (UNIT 2.5A). Resuspend in TE buffer at 1 ng/ul.

Carry out second PCR amplification. Combine the following in a 500-ul microcentrifuge tube:
10 ul (10 ng) each amplified fragment
1 ul (500 ng) each flanking sequence primer (each 1 uM final)
10 ul 10x amplification buffer
10 ul 2 mM 4dNTP mix
H_2O to 99.5 ul
0.5 ul Taq DNA polymerase (5 U/ul).

Overlay with 100 ul mineral oil. Carry out PCR for 20 to 25 cycles, using the conditions for introduction of restriction endonuclease sites by PCR (see Basic Protocol 1, step 6). Analyze and process the reaction mix (see Basic Protocol 1, Ausubel, supra, steps 7 and 8).

Digest the DNA fragment with the appropriate restriction endonuclease for the flanking sites (UNIT 3.1). Purify the digested fragment on a low gelling/melting agarose gel (UNIT 2.6). Subclone into an appropriately digested vector. Transform recombinant plasmid into E. coli (UNIT 1.8). Prepare DNA by plasmid miniprep (UNIT 1.6). Analyze the amplified fragment portion of the plasmid DNA by DNA sequencing (UNIT 7.4) to confirm the point mutation. This is critical because the Taq DNA polymerase can introduce additional mutations into the fragment (see Critical Parameters).

Example 6: hFGF-20 Induced Neurite Outgrowth in PC12 Cells

In order to directly determine the neurotrophic activity of FGF-20 compounds or compositions described in this invention, a neurite outgrowth assay can be performed with cells or cell lines derived from neuronal tissue essentially as described by Lyons et al., (1994). Briefly, PC12 cells are maintained at 37'C and 5% CO_z in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-

inactivated horse serum, 5% heat inactivated fetal bovine serum (FBS), and 1% glutamate. The cells are then plated at 105 per well in 96 well plates coated with 5 m/cm2 rat tail collagen and allowed to attach overnight. The medium is then replaced with DMEM, 2% heat-inactivated horse serum, 1% glutamate, 1-5 ng/ml of NGF (Sigma) and varying concentrations of the FGF-20 compound (0.001 nM - 100 μM). The background control culture is administered with 105 ng/ml of NGF alone without compound. Positive control culture's are administered with high concentration of NGF (50 ng/ml). The cells are then incubated at 37°C at 5% CO2 for 72 hours, fixed with 3% formaldehyde and nerve outgrowth can be determined visually and scored on a scale of 0 to 4. The assay should be done at least in triplicate for each concentration tested.

Example 7: hFGF-20 Induced Neurite Outgrowth in Human Neuronal SHSY-5Y Cells

Stimulation of neurite outgrowth by FGF-20 can also be demonstrated using the human neuronal SHSY-5Y cell line (ATCC). SHSY-5 Y cells are cultured and maintained in a 1:1 mixture of Eagle's Minimum Essential Medium and Ham's F-12 medium containing 10% feral bovine serum. For neurite outgrowth, cell are plated in chamber coverslips coated with Metrigel (Collaborative Research Inc.) at a density of 20,000 cells per chamber. FGF-20b (R&D Systems, Inc.) was added at various concentrations (0.001 nM - 100 μM). After 48 to 72 hours, cells were fixed with 5% Zn-formaline and stained with H&E stain. Neurite outgrowth was evaluated by phase contrast microscopy and can be scored as in Example 6. Neurite outgrowth can also be determined by measuring the expression of any known molecular marker of new axonal growth, including but not limited to the neuronal growth-associated protein 43 (GAP43) by any means know in the art.

GAP-43 is a phosphoprotein component of the neuronal membrane and growth cone that is selectively upregulated during new axonal growth in both the peripheral and central nervous systems. GAP-43 has previously been used as a reliable marker of new axonal growth during brain development, and following brain injury or ischemia (Kawamata et al., (1997(a)). Such axonal sprouting is likely to be accompanied by new dendritic sprouting and synapse formation in the intact uninjured brain. This in turn leads to enhanced recovery from neuronal damage as a result of disease of physical trauma. Immunostaining with axonal specific protein GAP43 followed by fluorescence microscopy according to known procedures shows that FGF-20 compounds resulted in a significant increase in neurite outgrowth over background control cultures (see Figure 2).

It will be clear that the present invention can be practiced otherwise than as particularly described in the foregoing description and examples.

Numerous modifications and variations of the present invention are possible in light of the above teachings and, therefore, are within the scope of the appended claims.

WE CLAIM:

1. An isolated polynucleotide comprising at least one hFGF-20 polynucleotide that is at least 95% identical over its entire length to a contiguous polynucleotide sequence within a polynucleotide sequence selected from the group consisting of:

a) SEQ ID NO:1;

b) SEQ ID NO:2; and

c) SEQ ID NO:3.

2. An isolated polynucleotide comprising a polynucleotide encoding polypeptide that is at least 95% identical over its entire length to a contiguous amino acid sequence within the a polypeptide sequence selected from the group consisting of

a) SEQ ID NO:4; and

b) SEQ ID NO:5.

3. An isolated hFGF-20 analog comprising the hFGF-20 polypeptide of Claim 3 wherein said polypeptide further comprises at least one substitution, insertion, or deletion selected from the group consisting of 5G, 9N, 10Y, 11F, 13V, 14Q, 15D, 16A, 17V, D18, D19, 20P, 21F, 23N, 24V, 25P, 26V, 29V, 50R, 53A, 54V, 55T, 56D, 58D, 61K, 78E, 80F, 82N, 84T, 85I, 90K, 94R, 103I, 123E, 135Q, 138V, 155L, 159V, 166Y, 177E, 179T, 181T, 198D, 199K, 207I, 209S, 210Q, and 211S of SEQ ID NO:4 or the corresponding amino acid of SEQ ID NO:5.

4. An isolated polynucleotide comprising a polynucleotide that is complementary to the polynucleotide of Claims 1-3.

5. A composition comprising the polynucleotide of claims 1-4 and a carrier or diluent.

6. A recombinant vector comprising the nucleic acid of claims 1-4.

7. A host cell comprising the recombinant vector of claim 6.

8. A method for producing a hFGF-20 polypeptide that comprises culturing the host cell of claim 7 under conditions such that the hFGF-20 polypeptide is expressed in detectable or recoverable amounts.

9. A transgenic or chimeric non-human animal comprising the polynucleotide of claims 1-4.

10. An isolated hFGF-20 polypeptide comprising a amino acid sequence selected from the group consisting of:

a) SEQ ID NO:4;

b) SEQ ID NO:5; and

c) a fragment of a) or b).

11. The isolated polypeptide of claim 10 further comprising a substitution, insertion, or deletion selected from the group consisting of 5G, 9N, 10Y, 11F, 13V, 14Q, 15D, 16A, 17V, D18, D19, 20P, 21F, 23N, 24V, 25P, 26V, 29V, 50R, 53A, 54V, 55T, 56D, 58D, 61K, 78E, 80F, 82N, 84T, 85I, 90K, 94R, 103I, 123E, 135Q, 138V, 155L, 159V, 166Y, 177E, 179T, 181T, 198D, 199K, 207I, 209S, 210Q, and 211S of SEQ ID NO:4 or the corresponding amino acid of SEQ ID NO:5.

12. An isolated polynucleotide probe or primer comprising having a sequence corresponding or complementary to at least 10 contiguous nucleotides of SEQ ID NOS:1, 2, or 3 and wherein said probe is able to hybridize to an hFGF-20 encoding mRNA transcript or fragment thereof.

13. An antibody that binds an epitope of the hFGF-20 polypeptide of claims 9-11.

14. A composition comprising a polypeptide of claims 9-11 and a carrier or diluent.

15. A composition comprising the antibody of claim 13 and a pharmaceutically acceptable carrier or diluent.

16. A method for enhancing neuronal growth, neurite outgrowth, neuronal regeneration, or neuronal survival in a mammal or in an *ex vivo* mammalian nerve cell comprising the step of administering to said mammal or said mammalian nerve cell an biologically effective amount of a composition according to claim 12.

17. A method for treating a human suffering from a neurological disorder comprising the administration of a therapeutically effective amount of a pharmaceutically acceptable composition of claim 14 or claim 15.

18. The method of claim 17 wherein said neurological disorder is selected from the group consisting of epilepsy, trigeminal neuralgia, glossopharyngeal neuralgia, Bell's Palsy, myasthenia gravis, muscular dystrophy, muscle injury, progressive muscular atrophy, progressive bulbar inherited muscular atrophy, herniated, ruptured or prolapsed invertebrae disk syndrome, cervical spondylosis, plexus disorders, thoracic outlet destruction syndromes, peripheral neuropathies caused by lead, dapsone, ticks, or porphyria, peripheral myelin disorders, Alzheimer's disease, Gullain-Barre syndrome, Parkinson's disease, Parkinsonian disorders, ALS, multiple sclerosis, central myelin disorders, stroke, ischemia associated with stroke, neural paropathy, neural degenerative diseases, motor neuron diseases, sciatic crush, neuropathy associated with diabetes, spinal cord trauma, facial nerve crush and other trauma, chemotherapy- or medication-induced neuropathies, and Huntington's disease.

19. A method for treating a human suffering from cancer comprising the step of administering to said human an effective amount of a pharmaceutically acceptable composition of claim 11.

20. The method of claim 19 wherein said cancer is of a type selected from the group consisting of melanoma, ocular melanoma, leukemia, astrocytoma, glioblastoma, lymphoma, glioma, Hodgkin's lymphoma, multiple myeloma, sarcoma, myosarcoma, cholangiocarcinoma, squamous cell carcinoma, CLL, and cancer of the pancreas, breast, brain, prostate, bladder, thyroid, lung, ovary, uterus, testis, kidney, stomach, colon or rectum.

21. Use of a polypeptide according to claim 10 or claim 11 for treating cancer.

22. Use of a polypeptide according to claim 10 or claim 11 for treating a neurological disorder.

23. Use of a FGF-20 agonist in the manufacture of a medicament for treating or preventing a neurological disorder, or at least one symptom associated therewith, in a mammal.

24. Use of a FGF-20 antagonist in the manufacture of a medicament for treating or preventing a neurological disorder, or at least one symptom associated therewith, in a mammal.

25. Use of a FGF-20 antagonist in the manufacture of a medicament for treating or preventing a cancer, or at least one symptom associated therewith, in a mammal.

26. The use of claim 23 wherein said FGF-20 antagonist is a FGF-20 antibody.

27. The use of claim 23 wherein said FGF-20 antagonist is a hFGF-20 human antibody.

X-13853

SEQUENCE LISTING

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<211> 211
<212> PRT
<213> Homo sapiens

<400> 4
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Leu Gly Gln Gln Val Gly Ser His Phe Leu Leu Pro Pro Ala Gly Glu
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Arg Pro Pro Leu Leu Gly Glu Arg Arg Ser Ala Ala Glu Arg Ser Ala
35 40 45

Arg Gly Gly Pro Gly Ala Ala Gln Leu Ala His Leu His Gly Ile Leu
50 55 60

Arg Arg Arg Gln Leu Tyr Cys Arg Thr Gly Phe His Leu Gln Ile Leu
65 70 75 80

Pro Asp Gly Ser Val Gln Gly Thr Arg Gln Asp His Ser Leu Phe Gly
85 90 95

Ile Leu Glu Phe Ile Ser Val Ala Val Gly Leu Val Ser Ile Arg Gly
100 105 110

Val Asp Ser Gly Leu Tyr Leu Gly Met Asn Asp Lys Gly Glu Leu Tyr
115 120 125

Gly Ser Glu Lys Leu Thr Ser Glu Cys Ile Phe Arg Glu Gln Phe Glu
130 135 140

Glu Asn Trp Tyr Asn Thr Tyr Ser Ser Asn Ile Tyr Lys His Gly Asp
145 150 155 160

Thr Gly Arg Arg Tyr Phe Val Ala Leu Asn Lys Asp Gly Thr Pro Arg
165 170 175

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Asp Gly Ala Arg Ser Lys Arg His Gln Lys Phe Thr His Phe Leu Pro
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Arg Pro Val Asp Pro Glu Arg Val Pro Glu Leu Tyr Lys Asp Leu Leu
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Met Tyr Thr
    210
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<210> 5
<211> 189
<212> PRT
<213> Homo sapiens

<400> 5

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Ser His Phe Leu Leu Pro Pro Ala Gly Glu Arg Pro Pro Leu Leu Gly
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Glu Arg Arg Ser Ala Ala Glu Arg Ser Ala Arg Gly Gly Pro Gly Ala
             20                  25                  30

Ala Gln Leu Ala His Leu His Gly Ile Leu Arg Arg Arg Gln Leu Tyr
         35                  40                  45

Cys Arg Thr Gly Phe His Leu Gln Ile Leu Pro Asp Gly Ser Val Gln
     50                  55                  60

Gly Thr Arg Gln Asp His Ser Leu Phe Gly Ile Leu Glu Phe Ile Ser
 65                  70                  75                  80

Val Ala Val Gly Leu Val Ser Ile Arg Gly Val Asp Ser Gly Leu Tyr
                 85                  90                  95

Leu Gly Met Asn Asp Lys Gly Glu Leu Tyr Gly Ser Glu Lys Leu Thr
            100                 105                 110

Ser Glu Cys Ile Phe Arg Glu Gln Phe Glu Glu Asn Trp Tyr Asn Thr
        115                 120                 125

Tyr Ser Ser Asn Ile Tyr Lys His Gly Asp Thr Gly Arg Arg Tyr Phe
    130                 135                 140

Val Ala Leu Asn Lys Asp Gly Thr Pro Arg Asp Gly Ala Arg Ser Lys
145                 150                 155                 160

Arg His Gln Lys Phe Thr His Phe Leu Pro Arg Pro Val Asp Pro Glu
                165                 170                 175

Arg Val Pro Glu Leu Tyr Lys Asp Leu Leu Met Tyr Thr
            180                 185
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